JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXXII WASHINGTON, D. C., JUNE 15, 1926 No. 12

OBSERVATIONS ON CIRPHIS LATIUSCULA H. SCH. IN THE GULF COAST REGION OF TEXAS[1]

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INTRODUCTION

Cirphis latiuscula (H. Sch.) is similar in its habits to two other well-known army worms, *Cirphis unipuncta* Haw., the common army worm, and *Cirphis albilinea* Hbn., the wheat-head army worm. It has been known in tropical America, especially in Cuba and Porto Rico, as one of the grass worms which feed on sugar cane and other large grasses, but it has not previously been recorded as injurious in the United States except in a short paper by the writer (*13, p. 389*)[2] in which are listed the parasites of this species found at Brownsville, Tex. The moths, however, have been reported from Texas, Arizona, and New Mexico.

In the sugar-cane fields at Brownsville, *Cirphis latiuscula* is the species commonly found, although an occasional specimen of *C. multilinea* Walk. may be taken. On oats and barley, *C. latiuscula* is commonly associated with *C. unipuncta* and more rarely with *C. multilinea* and *C. phragmitidicola* Guen. Along the Gulf coast it is nearly always necessary to rear the adults in order to be sure which species of Cirphis is causing damage, although one who is collecting them frequently learns to distinguish the larvae.

Although *Cirphis latiuscula* is commonly found in sugar-cane fields, it is not considered an important pest of sugar cane. Its significance as an economic insect lies in the fact that it is one of the species which may be concerned in army-worm outbreaks in the Southern States, especially along the Gulf coast. A knowledge of its seasonal history, habits, and parasites is, therefore, important as leading to a more complete understanding of the general problem of army-worm outbreaks.

SYSTEMATIC HISTORY

Cirphis latiuscula was described in 1868 by Herrich-Schäffer as *Leucania latiuscula* (*3, p. 148*) from specimens from Cuba. In 1877 Harvey (*2, p. 8*) described *Heliophila subpunctata* from specimens from Texas. Hampson in 1905 (*1, p. 542*) makes *subpunctata* Harvey a synonym of *latiuscula* Herr.-Schäff.

Hampson (*1, p. 542*) gives the synonymy of *Cirphis latiuscula* Herr.-Schäff.) as follows: *Heliophila subpunctata* Harv., *Leucania punctifera* Möschl., *L. senescens* Möschl., *L. tinila* Schaus, *L. com-*

[1] Received for publication Nov. 3, 1925; issued June, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 1118.

plicata Streck., *L. misteca* Schaus, *L. orizaba* Schaus, and *L. extincta* Druce (in part).

This moth has been known in the United States as *Heliophila subpunctata* Harvey and has been mentioned several times in the literature. Smith (*7, p. 189*) gives its habitat as Texas in March, and in 1902 he (*8, p. 162, 180*) described it and gave a figure of the male genitalia, specifying the habitat as Agricultural College, Miss., in October; Harris County and elsewhere in Texas in March; New Mexico near its southern border. Holland (*4, p. 201*) gives a figure of the female, and says that this species ranges from New Mexico and Texas to Arizona.

ECONOMIC HISTORY

Cirphis latiuscula has been mentioned several times in the economic literature as one of the grass worms of the American tropics. Stahl (*9, p. 193*) lists this species from Porto Rico. Van Dine (*11, p. 257*) records it as injurious to sugar cane in Porto Rico, and in his report for 1913 (*12, p. 34*) he says that the grass worm *C. latiuscula* H. Sch. eats young leaves of sugar cane. He mentions an undetermined braconid parasite and blackbirds as its natural enemies. Walton (*14, p. 93, 95*) describes *Compsilura oppugnator*, a parasite of *Cirphis latiuscula* reared at Rio Piedras, P. R., by T. H. Jones. Vickery (*13, p. 389*) records *Heliophila subpunctata* as injurious to sugar cane and grasses at Brownsville, Tex., and lists the parasites. Jones and Wolcott (*5, p. 43*) say that in Porto Rico this species is the least important of the noctuids whose larvae feed on cane leaves.

Generally speaking, it may be said that although this species has been mentioned numerous times in the systematic, faunistic, and economic literature no extended account of its habits and bionomics has been given.

HOST PLANTS AND DISTRIBUTION

The writer first observed the larvae of *Cirphis latiuscula* at Brownsville, Tex., on sugar cane October 9, 1910. Many of the sugar cane plants in the experimental plots of the South Texas Gardens [3] were severely injured by these larvae and by the larvae of *Mocis repanda* (Fab.). On October 15 a few larvae were found feeding on the leaves of corn, and on October 21 large numbers were discovered in a small patch of sorghum in which many plants had been completely defoliated.

From the fall of 1910 until 1917, when the work of the writer was discontinued in that locality, these larvae could be found in the sugar-cane fields at almost any time from October to April, inclusive. They were also frequently collected during March and April from the laboratory plots of barley and oats, where they were often very numerous.

The writer collected the larvae of *Cirphis latiuscula* only in the vicinity of Brownsville, Tex. C. L. Scott collected them from sugar cane at Riviera, and from Johnson grass at Victoria, Brazoria, and Sugarland, Tex.

[3] A plant-introduction garden maintained for several years by the United States Department of Agriculture on the Fort Brown Military Reservation, located on the Rio Grande adjacent to Brownsville, Tex.

HABITS OF THE LARVAE

The larvae of *Cirphis latiuscula* hide among the fallen dried leaves of their host plants or within the dried leaf sheaths at the base of the plant during the daytime, and feed only at night or during cloudy weather. In feeding they cling to the edge of the leaf with their legs and prolegs and grasp it on each side, pinching it between their mandibles and cutting notches out of it. By continuous feeding they make large smooth-edged notches. This type of injury is characteristic and distinguishes the damage done by *Cirphis latiuscula* and *Mocis repanda* from that caused by other larvae which may be found on the same host plants, such as the fall army worm, *Laphygma frugiperda* S. & A. and the bollworm, *Heliothis obsoleta* Fab., which have toothed mandibles with which they cut into the leaf surface. The two last-named species usually feed in the "throat" of young plants, such as corn or sorghum, and eat holes in the leaves. The holes made by Heliothis have a ragged edge, whereas those made by Laphygma have a smooth one.

In the sugar-cane fields the larvae of *Cirphis latiuscula* may be found in the fall, during the daytime, hiding within the dried leaf sheaths of the lower leaves of injured plants. If the leaves and tops of plants are left on the ground when the crop is harvested and remain there after the new cane sprouts come up in the spring, larvae may frequently be found in large numbers during March and April hiding under this trash during the daytime and feeding on the cane leaves at night.

Sorghum is grown for hay in southern Texas, the seed being sown broadcast, and these fields offer favorable conditions for the larvae, especially after one crop has been cut and leaves and stalks are left on the ground. The larvae of *Cirphis latiuscula* usually may be found in these fields in the fall.

In fields of barley and oats, after the plants have made a rank growth, many of the lower leaves die and fall to the ground, affording cover for large numbers of larvae. Army worms may nearly always be found in such situations during March and April along the Gulf coast of Texas wherever these crops are grown, but they are not extensively cultivated in that region. Fields of grass such as *Panicum fasciculatum* also afford ideal conditions for the larvae. The larvae are seldom present in cultivated crops such as corn or even in cane fields where the trash is burned, although they may migrate to any grass crop from fields where they have become numerous.

In the lower Rio Grande Valley, the larvae of *Cirphis latiuscula* were nearly always found in company with other injurious species. When *Mocis repanda* is numerous *C. latiuscula* is usually associated with it. On barley and oats, however, it is generally associated with *C. unipuncta*. On April 4, 1917, the writer collected many army worms from oats, and from these were reared four species: *C. latiuscula*, *C. unipuncta*, *C. multilinea*, and *C. phragmitidicola*.

REARING EXPERIMENTS

Experiments with *Cirphis latiuscula* were retarded by the difficulty of getting unparasitized material at favorable times, and by obstacles encountered in rearing the larvae under observation and in getting the moths to lay eggs freely.

The moths were placed in large street-lamp globes, and were fed on thin sugar sirup. At first green corn leaves were placed in the cages, but the moths laid very few eggs on the green leaves; so dry leaves were used instead, and they deposited eggs more freely. The eggs were laid between two leaves, or in a fold of the leaf.

Strips of paper were later placed in the cages and the moths oviposited on these very freely. The paper was cut into strips 4 by 9 inches, folded lengthwise, and stacked in the cages with the folded edges toward the center so that the outside surfaces of the strips were in contact. The moths laid their eggs between the strips of paper, usually near the edge. The eggs were attached to both strips of paper, but more lightly to one than to the other, so that it was easy to remove them by separating the strips and cutting out the paper where the masses were attached.

Eggs, larvae, and pupae were kept in seamless tin boxes. Larvae collected for the purpose of rearing parasites were isolated in 1-ounce boxes. For studying the larval stages many were kept together in a 2-ounce box until after the third molt, when they were isolated. Parasites were kept in large vials.

SEASONAL HISTORY

There is no hibernating stage for *Cirphis latiuscula* in southern Texas, all stages being found in the field during the winter; nor is there a definite number of generations, for the generations overlap and all stages may be found in the field during any month of the year.

The entire life cycle, from the time the eggs are laid until the moths emerge, requires from 40 to 50 days during the months from May to September, inclusive; from 50 to 70 days during October, November, March, and April, and more than 70 days during December, January, and February. There are probably about six generations during the year.

Larvae were collected during every month except August. They are easily found in the sugar-cane fields during October, November, and December, or until the cane is cut in January. During February, March, and April they may be taken on oats and barley. During March and April they may also be taken in the sugar-cane fields, especially in the fields where the trash is left unburned. During the summer months they may be found in sugar-cane or sorghum fields, or feeding on grasses.

The larvae are numerous from October to April, but are difficult to find during the summer. The reason for this is not evident, but it may be that their numbers are reduced by parasites during March and April, or that there is so much vegetation from May to August that the presence of the larvae is not noticeable.

EGG STAGE

The eggs are laid in masses of one layer, in long, nearly regular rows, four or five rows wide. They are usually in contact in the rows, and the rows also are in contact, or when not in contact they are connected by flattened bands of cement. The eggs are deposited between two leaves, or in a fold of a leaf, on their sides with the micropyle toward the opening.

Eggs were not found in the field, but it is probable that they are laid in the trash on the ground in the fields of young cane, or between the dry leaves at the base of older cane plants. In fields of grass or grain they probably are laid on the lower dead leaves.

INCUBATION PERIOD

The incubation period was determined accurately in only a few instances. Eggs laid the night of June 25–26, 1914, hatched the night of June 29–30, the incubation period being 4 days at an average temperature of 84° F. Eggs laid the night of December 3–4, 1915, hatched at noon December 12, the incubation period being 8 days. In this case the eggs were kept in the office and the exact temperature was not recorded, but it was about 70° F. in the daytime.

Eggs which were laid November 27 to November 29, 1915, were placed in an ice box on November 30, and on December 17 two larvae hatched, the incubation period being about 18 days at an average temperature of about 55° F. The temperature of the ice box was above 55° F. for only a few days and did not go higher than 62° F.

Under natural conditions at Brownsville the incubation period would probably vary between 4 and 8 days from April to October, and from 8 to 20 days during the colder months from November to March.

LARVA STAGE

Only one record was obtained of the development of the larva from egg to pupa. The eggs hatched May 14, 1914. The larvae were fed on corn leaves and began to pupate on June 10, thus making the larva stage 27 days long. The mean temperature in the laboratory during this period was 78° F.

PUPA STAGE

Table I shows some of the records for the pupa stage made from 1910 to 1915 at Brownsville. The shortest period was 9 days for two males in June at a mean temperature of 83° F., and the longest was 23 days in November and December at a mean temperature of 66° F.

TABLE I.—*Pupation records of Cirphis latiuscula made at Brownsville, Tex., 1910 to 1915*

Date larva pupated	Date adult emerged	Length of pupa stage	Mean temperature	Number and sex of adults
		Days	*° F.*	
Mar. 10–12, 1915	Apr. 1, 1915	21	65	1[a].
Apr. 4, 1913	Apr. 22, 1913	18	73	1[a].
Apr. 15, 1911	Apr. 28, 1911	13		1[a].
June 10, 1914	June 20, 1914	10	82	3 females.
June 11, 1914	June 21, 1914	10	82.5	1 female.
Do	June 22, 1914	11	83	2 females.
June 12, 1914	June 21, 1914	9	83	1 male.
Do	June 22, 1914	10	83	Do.
Do	do	10	83	1 female.
June 13, 1914	do	9	83	1 male.
June 15, 1914	June 25, 1914	10	83	1 female.
Oct. 8, 1914	Oct. 20, 1914	12	76	3[a].
Do	Oct. 21, 1914	13	76	2[a].
Oct. 26, 1910	Nov. 9, 1910	14		1[a].
Do	Nov. 10, 1910	15		1[a].
Do	Nov. 12, 1910	17		1[a].
Nov. 14, 1914	Dec. 7, 1914	23	66	2[a].

[a] Sex unknown.

The length of the pupa stage at Brownsville under natural conditions would be from 9 to 13 days during the months from May to September, inclusive; from 14 to 23 days during October, November, March, and April; and 23 days or more during the winter months December, January, and February. There are always warm nights during the winter months on which moths may emerge.

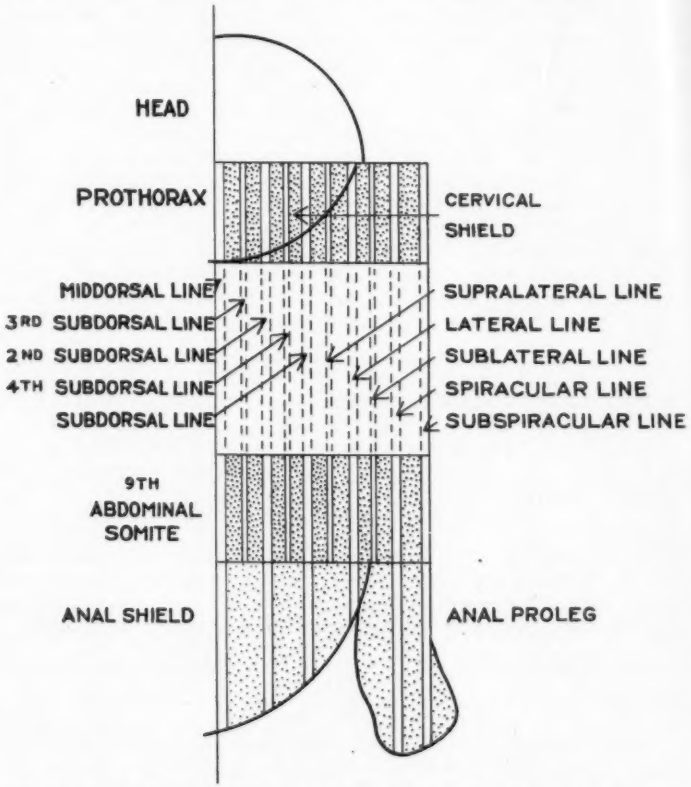

FIG. 1.—Diagrammatic representation of one side of a caterpillar of *Cirphis latiuscula*, from middorsal line to the subspiracular line, spread flat, to show the terminology of the lines used in the descriptions of the stages.

DESCRIPTION OF THE DIFFERENT STAGES

THE EGG

The egg is spherical, smooth, flattened on one or more sides when in contact with other eggs or with the object upon which it is laid; and 0.5 mm. in diameter. Under high power the upper surface appears finely reticulated, being divided into small hexagonal areas which radiate from the micropyle. Color: Lustrous, straw-yellow when first laid, changing to warm buff and to ocherous buff during developmeent, and to slate just before hatching.

COLOR OF THE LARVAL STAGES

Figure 1 gives the terminology of the lines used in the descriptions of the stages. The lines are not recognizable in the newly-hatched larvae, but they develop as the larva grows and molts until the fourth stage, when all lines

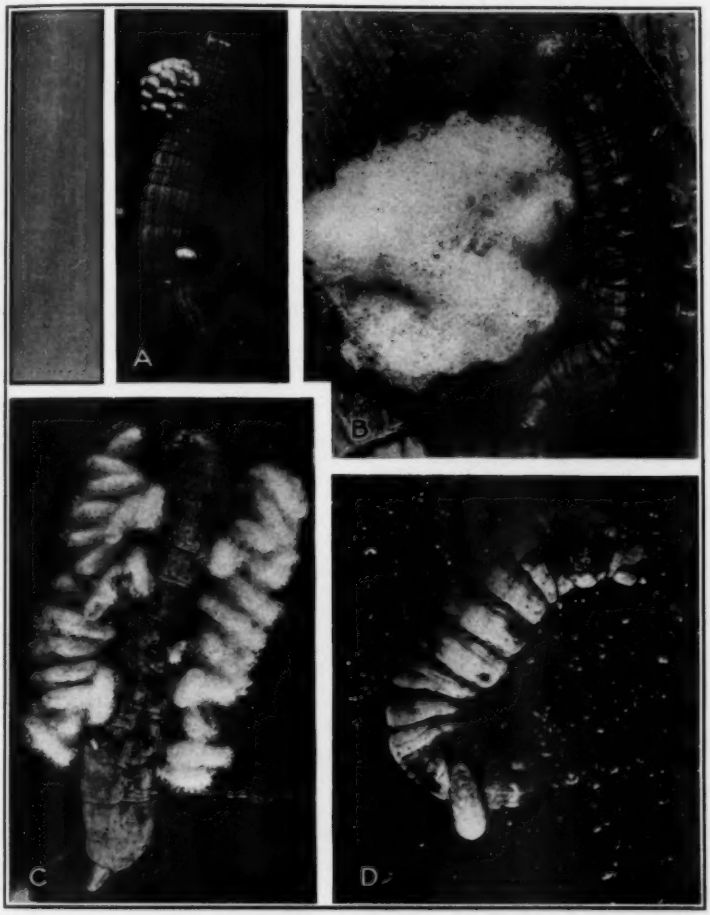

FIG. 2.—Parasites of *Cirphis latiuscula:* A, caterpillar of *C. latiuscula* in the fifth stage attacked by a colony of the larvae of *Euplectrus platyhypenae* (this cut shows the characteristic appearance of the caterpillar); B, caterpillar of *C. latiuscula* in the last stage killed by *Apanteles rufocoxalis*, the larvae of which have emerged and spun their cocoons in a mass near the caterpillar (this also shows the sepia reticulations on the head of the caterpillar and the sepia reticulations and mottlings above the spiracular line and above the subdorsal line); C, caterpillar of *C. latiuscula* in the last stage killed by *Apanteles militaris* the larvae of which have emerged and have spun their cocoons on the caterpillar; D, caterpillar of *C. latiuscula* in the last stage, attacked by a larva of *Paniscus* sp.

are recognizable, at least in some specimens. In the fifth and sixth stages the lines are not so distinct, as they then tend to break up into mottlings.

The pale lines are numerous and close together, giving the caterpillar a striated appearance to the naked eye (fig. 2, A). There is one middorsal line, and on

each side are five other primary lines; the spiracular and subspiracular are closer together than the others, the lateral just touching the cervical shield on the side; the subdorsal is about one-third the distance between the lateral and the middorsal; the second subdorsal is midway between the middorsal and subdorsal. The middorsal, subdorsal, second subdorsal, and lateral lines extend onto the cervical shield and onto the anal shield. The spiracular and subspiracular lines extend onto the anal prolegs.

On each side there are four secondary pale lines; the third subdorsal between the second subdorsal and the middorsal; the fourth subdorsal between the second subdorsal and the subdorsal; the sublateral and supralateral, respectively, below and above the lateral line. All of the secondary lines except the sublateral line extend onto the cervical shield, but they do not extend onto the anal shield.

First Stage

Head: 0.25 to 0.3 mm. wide, uniform cream color.

Body: About 3 mm. long and 0.3 mm. wide, translucent or whitish, sometimes greenish from food eaten, sayal brown color (*6*) beginning to show in places. Cervical shield brown, cornified. First pair of prolegs short, second pair a little longer. In this stage the caterpillars are semiloopers, using only the last three pairs of prolegs in locomotion.

Second Stage

Head: 0.4 to 0.55 mm. wide, cream color, sepia reticulations beginning to appear on the epicrania.

Body: 3 to 6.5 mm. long, 0.4 to 0.6 mm. wide; cervical shield slightly cornified; middorsal, subdorsal, and lateral lines distinct, ivory yellow in color; general color cream,[4] sometimes greenish from food eaten; faint sayal brown mottlings dorsad of the spiracular lines, strongest between the subdorsal and the spiracular lines and divided by the lateral line so as to form two sayal brown stripes on each side. Legs cream color.

Third Stage

Head: 0.75 to 0.8 mm. wide, cream color, sepia reticulations on epicrania.

Body: 0.8 to 10 mm. long; 0.9 to 1.4 mm. broad; cervical shield slightly cornified, with sepia pigment. All primary lines rather broad and well defined, varying in color from ivory yellow to sea-foam green (*6*), except the lateral line, which is whiter and not so bright; the second subdorsal line is broad and not well defined, without pigment. The general color of the dorsum is sayal brown, slightly darker on each side between the lateral and the spiracular lines. Venter ivory yellow; legs ivory yellow. This stage has all of the primary lines, and, in addition, some of the secondary lines are faintly indicated. The pigmentation of the primary lines is very little interrupted. The first pair of prolegs is not so well developed as the other prolegs but they are used in locomotion.

Fourth Stage

Head: 1.1 to 1.2 mm. wide, cream color, epicrania with sepia reticulations.

Body: 8 to 13 mm. long, 1.1 to 1.4 mm. wide; cervical shield pale olive buff, slightly cornified; anal shield pale olive buff. All primary lines well defined, broad, greenish-yellow in color; lateral line whiter than the others; the second subdorsal line outwardly irregular on the cervical shield. The secondary lines are faintly indicated, the sublateral distinct on the prothorax; the fourth subsubdorsal and the supralateral not developed, or indistinct. When the larva is ready to molt the third subdorsal is almost as distinct as the second subdorsal, the two together forming a double line on each side between the middorsal and subdorsal. The venter ivory yellow; dorsum sayal brown, but this color is not uniform, being in the form of sayal brown mottlings on the ivory-yellow ground color. When the alimentary canal is full of food a greenish cast is given to the general color. Between the spiracular line and the lateral line on each side the sayal brown color is more uniform and is darker, forming a sepia stripe on each

[4] This refers to the color of the caterpillar as it appears to the naked eye or with a low-power hand lens. The details of color such as appear in the lines, mottling, and reticulations may be observed with a high-power hand magnifier or with a low-power binocular microscope.

side dorsad of the spiracles. Legs ivory yellow, translucent; shields on the legs and prolegs transparent; all prolegs equally well developed, translucent; anal prolegs pale olive buff.

Fifth Stage

Head: 1.5 mm. wide, cream color, epicrania with sepia reticulations (fig. 2, B) and a sepia stripe bordering the paraclypeal pieces and extending to the caudal part of the head, parallel with and near to the median suture; front with a median vertical dash of sepia; labrum thin, transparent; mandibles brown, with a wide black border along the cutting edge; ocelli sepia or black; no tubercles; setae short and transparent.

Body: Cervical shield not cornified; tubercles inconspicuous; setae short and transparent; a small black circle around the bases of setae, and sometimes a slight dark shading near this. The striated appearance is not so marked in this stage. The primary lines are sea-foam green in color. Middorsal line narrow and not very distinct, subdorsal line broader and more distinct, lateral line broad but not well defined, spiracular line just traceable; all of these lines well defined on the prothorax. Secondary lines indistinct or not traceable. General color sayal brown, sepia reticulations (fig. 2, B) on each side between the spiracular and lateral line, and on the dorsum between the subdorsal lines. The mottlings ventrad of the subspiracular are white, and dorsad of the subspiracular they are of a warm buff color and close together. These mottlings are set off by the sepia color where there are sepia reticulations.

Sixth, Seventh, and Eighth Stages [5]

Head: 3.2 mm. wide; in other respects similar to fifth instar.

Body: 29 mm. long, 4.2 mm. wide; general color sayal brown or tawny olive; cervical shield not cornified. Middorsal line narrow; subdorsal line faint or sometimes broad and distinct; lateral line usually not well defined; spiracular line broken or diffused, made up of mottlings of sea-foam green; subspiracular line only faintly indicated. The secondary lines are broken and diffused, made up of mottlings of sea-foam green. The body is marked with fine mottlings of several colors, sepia, tawny olive, sayal brown, cinnamon, and lighter mottlings of sea-foam yellow (*6*) or sea-foam green. The darker mottlings occur on the dorsum between the subdorsal lines; they are always heaviest just dorsad of the subdorsal lines, forming a sepia stripe on each side between the subdorsal and second subdorsal. The darker mottlings also occur between the lateral and spiracular lines, forming a sepia stripe on each side just dorsad of the spiracles. The darker mottlings are absent between the subdorsal and lateral lines, forming a lighter colored subdorsal stripe; and ventrad of the spiracles forming a similar subspiracular stripe. The ground color is ochraceous orange. Venter light olive with few mottlings.

THE ADULT

The following description is taken from Smith (*8, p. 180*):

Color a dull, grayish red-brown over a yellowish base. Head with two darker transverse lines on front. Collar with three lighter and three smoky lines alternating. The patagiae are much powdered with blackish. Primaries with a dark smoky or blackish shade over the median vein, relieving a white dot at its end and extending beyond it nearly to the transverse posterior line. In the costal region the veins are whitish, the interspaces streaked with blackish. Along the inner margin is a paler, more yellowish area in which the streakings are lighter. The terminal area is dark, limited above by a somewhat yellowish oblique subapical shade. Transverse posterior line punctiform, complete. Secondaries white, somewhat translucent, veins and outer border smoky. Beneath primaries smoky with a reddish tinge; a black spot on costa toward apex from

[5] The writer was not able to rear any single larva through all of the stages and record an exact description for each stage, because the larvae did not do well when handled frequently. When reared in confinement they passed through about eight stages. As all of the stages from the sixth to the eighth are similar, except for the width of the head, only six stages are described. The width of the head is a distinguishing character up to and including the fourth stage, but the first five stages may be distinguished by other characters. In the last stage the width of the head is about 3.2 mm., but the writer does not know how many molts any single larva passes through in order to reach that size.

which a smoky line may extend across the wing. Secondaries whitish, with the costal and outer margins more or less obviously reddish gray, powdery.

Expanse: 1.35 to 1.52 inches (34 to 38 mm.).

PARASITES OF CIRPHIS LATIUSCULA

METHODS USED IN COLLECTING AND REARING PARASITES

Many caterpillars were collected in the sugar-cane fields during the daytime by searching in the trash on the ground near injured young plants, or inside the lower dried leaf sheaths of old plants. Larvae were most easily found along the irrigation ditches or at the edges of fields. A few collections were made at night with a flash light. The caterpillars may be observed and collected while they are feeding at night, especially on young sugar-cane plants in April or May.

Larvae were collected from oats and barley in the daytime by searching under the matted dried lower leaves which covered the ground. They were also taken from these plants at night with a sweep net while they were feeding. Collections from barley or oats nearly always included *Cirphis unipuncta*, and occasionally *C. multilinea* or *C. phragmitidicola*.

After the caterpillars had been collected and brought to the laboratory they were sorted into species, *C. unipuncta* and *C. latiuscula*, and each species was sorted into two classes, large larvae, including those in the fifth and sixth stages, and small larvae, including those in the fourth stage or earlier. Each larva was then isolated in a 1-ounce tin box.

All larvae were examined and fed every day or every other day. They were given leaves of barley or leaves of corn. The leaves were wiped with cloth to remove tachinid larvae or eggs, and cut into short lengths to fit the boxes.

After being used, the boxes were wiped clean, washed, and then sterilized by dry heat in an oven over a gasoline stove. As soon as the larvae pupated the pupae from each lot were placed together in larger boxes, and after several days those containing tachinid puparia were removed and placed in large vials so that the emergence of the tachinids could be observed easily. The parasites which emerged from the larvae were placed in vials as soon as the cocoons were spun.

TABULATION OF THE RESULTS OF PARASITE REARINGS

From November 27, 1912, to November 6, 1915, numerous collections [6] of *Cirphis latiuscula* were made, totaling 1,582 caterpillars, and these were reared in the laboratory for the purpose of gaining a knowledge of parasitism in relation to this species. The information obtained from these collections is summarized in Tables II, III, and IV.

Table II gives a detailed tabulation of the collections showing the date, locality, number of larvae collected, and the species of parasites reared.

[6] Many of the collections were made by C. L. Scott of the Bureau of Entomology, United States Department of Agriculture, but the parasites were reared and studied by the writer.

TABLE II.—*Number of parasites reared from larvae of Cirphis latiuscula collected in the vicinity of Brownsville, Tex., from November 27, 1912, to November 6, 1915*

Locality in which collection was made	Host plant	Date of collection	Number of larvae collected	Number of parasites reared									Number of larvae that died from—		Number of moths that emerged
				Hymenoptera						Tachinidae		Total number of parasites reared			
				Apanteles militaris	*Apanteles rufocoxalis*	*Euplectrus platyhypenae*	*Meteorus laphygmae*	*Paniscus* sp.	*Microplitis varicolor*	*Peleteria robusta*	*Archytas pilicentris*		Fungus *Beauveria globulifera*	Injury or disease	
Brownsville	Cane	Sept. 26, 1915	19												19
San Benito	do	Oct. 14, 1914	20	1							2	3			17
Brownsville	do	Oct. 30, 1914	25	1		1					1	3			22
Do	do	Oct. 26, 1915	6	1							1	2		2	2
Do	do	Oct. 30, 1915	30	6		1						7		6	17
Harlingen	do	Nov. 27, 1912	58	3		2	2				2	9			49
Brownsville	do	Nov. 5, 1914	45	5				2			6	13			32
Do	do	Nov. 6, 1914	18	2	1							3			15
Do	do	Nov. 10, 1914	42	5	4						9	18	1		23
Do	do	Nov. 10, 1914	45	9	3		1					13			32
Harlingen	do	Nov. 17, 1914	89	47		2						49			40
Donna	do	Nov. 18, 1914	24	4							1	5			19
Harlingen	do	Nov. 21, 1914	80	32		1	1				9	43		5	32
Brownsville	do	Nov. 6, 1915	49	1	4						7	12			37
Harlingen	do	Dec. 4, 1914	90	39	1	2	1	1			8	52		10	28
Do	do	Jan. 12, 1915	18	4	5							9		3	6
Brownsville	Oats	Feb. 23, 1915	15	4			1					5	1	3	6
Do	do	Mar. 13, 1915	74	13			2					15	3	35	21
Do	do	Mar. 23, 1915	87	30	10	1	5		1			47		15	25
Do	do	Mar. 23, 1915	22	8								8	1	6	7
Do	Cane	Mar. 24, 1915	64	32	3		5	2				42		16	6
San Benito	do	Mar. 30, 1915	13	6								6		6	1
Do	do	Mar. 30, 1915	7	1	3							4		2	1
Brownsville	do	Mar. 31, 1915	13	4	3	1		1				9		3	1
Do	Oats	Mar. 31, 1915	5	3		1						4			1
Do	Cane	Apr. 2, 1914	12		3	2		1		2	1	9			3
Do	do	Apr. 4, 1914	58		6			3		8	17	34	3	11	10
Do	do	Apr. 10, 1914	40		5	1		4		5	13	28		10	2
Do	do	Apr. 15, 1914	46		24	3		5		1	6	39	2	5	
Do	Barley	Apr. 18, 1914	92	20	9	1	4		3		2	39	1	18	34
Do	Cane	Apr. 1, 1915	6	4								4		2	
Do	do	Apr. 2, 1915	181	101	13	13	1	9			8	145		18	18
Do	do	Apr. 2, 1915	42	10	1		2				1	14		17	11
Donna	do	Apr. 8, 1915	47	14		1						15		16	16
Brownsville	do	Apr. 22, 1915	100	17	48	5		1				71	2	18	9
			1,582	427	146	38	25	29	4	16	94	779	14	227	562

Table III is based on Table II and shows the number of caterpillars collected in each month and the percentage of each species of parasite reared.

TABLE III.—*Percentage of caterpillars of Cirphis latiuscula infested with parasites reared from collections made in the vicinity of Brownsville, Tex., November 27, 1912, to November 6, 1915*

Month in which collection was made	Number of larvae collected	Percentage of parasites reared									Percentage of larvae that died from—		Percentage of moths emerged
		Hymenoptera						Tachinidae		Total percentage of parasites reared			
		Apanteles militaris	*Apanteles rufocoxalis*	*Euplectrus platyhypenae*	*Meteorus laphygmae*	*Paniscus* sp.	*Micropilitis varicolor*	*Peleteria robusta*	*Archytas piliventris*		Fungus *Beauveria globulifera*	Injury or disease	
September	19												100
October	81	11		2					5	19		10	72
November	450	24	3	1	1	0.4			8	37	0.2	1	62
December	90	43	1	2	1	1			9	58		11	31
January	18	22	28							50		17	33
February	15	27			7					33	7	20	40
March	285	34	7	1	4	1	0.4			47	1.4	29	22
April	624	27	17	4	1	4	.5	2.5	8	64	1.3	18	17
Total	1,582	27	9	2	2	2	.3	1	6	49	1	14	36

Table IV is also based on Table II, and shows the collections of April, 1914, April, 1915, and November, 1914, arranged for comparison.

TABLE IV.—*Percentage of parasites reared from larvae of Cirphis latiuscula collected in the vicinity of Brownsville, Tex., in April, 1914, April, 1915, and November, 1914, arranged by percentages for comparison*

Date of collection	Number of larvae collected	Percentage of parasites reared									Percentage of larvae that died from—		Percentage of moths emerged
		Hymenoptera						Tachinidae		Total percentage of parasites reared			
		Apanteles militaris	*Apanteles rufocoxalis*	*Euplectrus platyhypenae*	*Meteorus laphygmae*	*Paniscus* sp.	*Micropilitis varicolor*	*Peleteria robusta*	*Archytas piliventris*		Fungus *Beauveria globulifera*	Injury or disease	
April, 1914	248	8	19	3	2	5	1	6	16	60	2	18	20
April, 1915	376	30	16	5	1	3			2	66	1	19	14
November, 1914	343	30	2.3	1	.6	.6			7	42	.3	1.5	56

DISCUSSION OF DATA ON PARASITES

Of all larvae collected, 295 were taken from oats and barley, and 1,287 from sugar cane. The following figures, based on the data given in Table II, show the percentages of parasites reared from *C. latiuscula* larvae collected on oats and barley: *Apanteles militaris* 26 per cent, *A. rufocoxalis* 6, *Euplectrus platyhypenae* 1, *Meteorus*

laphygmae 4, *Paniscus* sp. 0, *Microplitis varicolor* 1, *Peleteria robusta* 0, *Archytas piliventris* 1; total, 40 per cent. Two per cent were killed by the fungus *Beauveria globulifera*, and 26 per cent died from injury or disease. Moths were reared from 32 per cent of the larvae. The following figures, also based on the data in Table II, show the percentages of parasites reared from *C. latiuscula* larvae collected from sugar cane: *A. militaris* 27 per cent, *A. rufocoxalis* 10, *Euplectrus platyhypenae* 3, *Meteorus laphygmae* 1, *Paniscus* sp. 2, *Microplitis varicolor* 0, *Peleteria robusta* 1, *Archytas piliventris* 7; total parasites, 51 per cent. One per cent was killed by the fungus *Beauveria globulifera*, and 12 per cent died from injury or disease. Moths were reared from 36 per cent of the larvae.

Comparing the total number of larvae obtained from oats and barley (295) with the total number from sugar cane (1,287), it appears that the total percentage of parasites reared was much greater in the larvae from sugar cane. *Apantales militaris* destroyed about the same percentage (26 per cent) of the larvae on oats and barley as it did on sugar cane. All other species of parasites together destroyed 24 per cent of the larvae from sugar cane, as compared with 13 per cent from oats and barley. The percentage was greater for all species, except *Meteorus laphygmae*, which is usually more numerous on low-growing plants, and *Microplitis varicolor*, which was not reared from larvae collected from sugar cane. *Archytas piliventris* was much more numerous on larvae collected from sugar cane. *Paniscus* sp. and *Peleteria robusta* were reared only from larvae collected from sugar cane.

More than twice as many larvae died from disease or injury in the collections from oats and barley as died from these causes in the collections from sugar cane.

Generally speaking, the same species of parasites attack *Cirphis latiuscula* caterpillars when they are feeding on oats or barley as attack them when they are feeding on sugar cane.

Comparing collections made during November, 1914, with those made during April, 1915 (Table IV), we find a total parasitism of 66 per cent in April as compared with 42 per cent in November. The increase of 24 per cent in the spring was caused by an increase of all species represented except *Archytas piliventris*, which showed a loss of 5 per cent, but the most important increases were those in *Apantales militaris* and *A. rufocoxalis*. Only 1.5 per cent of the larvae were killed by unidentified causes in November as compared with 19 per cent in April, so that the percentage of parasitism was probably greater in the spring than is shown here.

Usually, larvae collected in the spring were more heavily parasitized than those collected in the fall.

Collections made in April, 1914, and April, 1915 (Table IV), were numerous enough to make possible a comparison between the parasitism of the two years in this month. One important difference was the large number of tachinids in 1914. Tachinids destroyed 22 per cent of the larvae in 1914 as compared with 2 per cent in 1915. *Archytas piliventris* emerged from 16 per cent of the larvae in April, 1914, as compared with 2 per cent in April, 1915. *Peleteria robusta* emerged from 6 per cent of the larvae collected in April, 1914, but this species was not reared in 1915. This increased efficiency of the

tachinids in 1914 was offset by the decrease in *Apanteles militaris*, which parasitized only 8 per cent of the larvae collected in April, 1914, as compared with 39 per cent in April, 1915. It appears from this that the efficiency of the various species of parasites may vary from year to year.

Apanteles militaris Walsh [7]

Of all the parasites of *Cirphis latiuscula* in the lower Rio Grande Valley, the most effective is *Apanteles militaris* (fig. 2, C), which was reared from 27 per cent of all larvae collected. There was little difference in percentage of infestation between larvae collected from sugar cane and those collected from barley and oats. *A. militaris* was reared from larvae taken from sugar cane in October, November, December, January, March, and April; and from larvae taken from barley or oats in February, March, and April. It appeared to be most effective in March, when 34 per cent of the comparatively large number of larvae collected were infested by this species.

These parasites emerge in large numbers from full-grown or nearly full-grown caterpillars, and spin their white cocoons in masses side by side standing on end. The group of cocoons is not covered with a cottony mass of silk like that of *Apanteles rufocoxalis*, and each cocoon is visible. The number of cocoons spun on 19 larvae in the fifth and sixth stages ranged between 9 and 132, with an average of 45.

Oviposition by this species and by *Apanteles rufocoxalis* was observed in the laboratory. It is accomplished in the same manner by both species. The female parasite attacks the caterpillar as soon as she encounters it, and she oviposits in any available part of the larva. She thrusts in her ovipositor and holds it there a few seconds. The victim turns and strikes at the parasite with its head, and usually soon frees itself. Oviposition was not observed in the field, but it is the opinion of the writer that *Apanteles militaris* and *A. rufocoxalis* are both diurnal, and that they oviposit in the host caterpillars while they are hiding under the cane trash in the daytime.

Many individuals of *Apanteles militaris* were placed in a cage with caterpillars of *Cirphis unipuncta* on April 18, 1915. The cage was a large wooden tray covered with glass. Apanteles colonies emerged from 8 larvae on April 28, and from 29 larvae on April 29. The egg and larval periods together required 10 days at an average temperature of 76° F. The details of the larval life of *A. militaris* have been described by Tower (*10*).

The pupa stage of *Apanteles militaris* appears to be of short duration.

A few records made at Brownsville, Tex., are given in Table V.

[7] The writer acknowledges his indebtedness to A. B. Gahan, R. A. Cushman, and W. R. Walton, of the Bureau of Entomology, United States Department of Agriculture, for the determination of the parasites.

TABLE V.—*Pupa stage of Apanteles militaris at Brownsville, Tex., March 6, 1913, to March 5, 1915*

Date cocoons spun	Date adults emerged	Number of days in pupa stage	Temperature
1915	1915		° *F.*
Feb. 25..	Mar. 5..	8	67
1913	1913		
Mar. 6..	Mar. 19..	13	65
Mar. 18..	Mar. 24..	6	71
Mar. 21..	Mar. 27..	6	72
Mar. 24..	Apr. 2...	9	72
Apr. 15..	Apr. 22..	7	73

The cocoons of *Apanteles militaris* may be found in the field under cane leaves on the ground, or under the dried lower leaves of barley or oats. Large numbers of these cocoons were collected and two species of parasites, *Gelis minimus* (Walsh) and *Eupteromalus viridescens* (Walsh), were reared from them.

Gelis minimus is the most common parasite of *Apanteles militaris*. In March, 1915, oviposition by this species was observed in the laboratory. A female was placed in a vial with unparasitized cocoons. She crawled over them exploring them with her antennae, and when she seemed to find a favorable location she placed the point of her ovipositor in contact with the cocoon and then withdrew the ovipositor from its sheath and pressed it into the cocoon. She withdrew it partly and thrust it in again. She remained about two minutes on one cocoon but not so long on others.

One unmated female Gelis was placed in a vial with cocoons of *Apanteles militaris* on March 8, 1915, and two males emerged on March 30; another unmated female was placed with cocoons on March 12, and two male Gelis emerged on March 30, 1915. In another case females oviposited in fresh cocoons on March 22, and two males emerged from the cocoons on April 10, 1915. In these cases the total life history required 18 to 22 days. The experiments were conducted in the office where the temperature was around 70° F., but no exact record of the temperature was kept. Parasites of this species did not mate in the vials in which they were kept. and no experiments were conducted with fertilized females.

Eupteromalus viridescens is also a common parasite of *Apanteles militaris*. Two unmated females of this species were placed in a vial with new cocoons of Apanteles on February 28, 1915, and left there for 36 hours. Thirteen male adults emerged from these cocoons on March 16, the time from oviposition to the emergence of the parasites being 16 days. In another experiment, unmated females oviposited in fresh cocoons on March 7, 1915, and on March 28 thirteen parasites emerged, the time from oviposition to the emergence of the adults being 21 days. These parasites also were kept in the office where the temperature was 70° F. or above.

Apanteles rufocoxalis Riley

Next to *Apanteles militaris* the most important parasite of *Cirphis latiuscula* is *A. rufocoxalis* Riley (fig. 2, B), which was reared from 9 per cent of all larvae collected. These larvae were taken from sugar cane in November, December, January, March, and April; and from barley or oats in March and April. It was most effective in April, especially in sugar-cane fields.

These parasites also emerge as larvae from full-grown or nearly full-grown caterpillars, and spin their cocoons in a nearly circular mass, partly on the dead caterpillar and partly on the leaf or other object upon which it rests. The cocoons stand on end side by side and the entire group is covered with a cottony mass of silk so that it is difficult to distinguish the individual cocoons. The cocoons of this species may be found in the cane fields under the fallen dried leaves or under the dead lower leaves of barley or oats. Large numbers were collected for the purpose of rearing parasites, but none was reared. The silk protection spun over the mass of cocoons may protect them from parasites.

Cocoons of *Apanteles rufocoxalis* were spun between February 27 (1915) and March 1, and the adult parasites emerged on March 12. The time in the cocoon was 12 days, at an average temperature of 64° F.

On April 21, 1915, male and female *Apanteles rufocoxalis* were placed in a large vial with reared larvae of *Cirphis unipuncta* which had heads 2.2 mm. wide and were ready to molt. The parasites oviposited in the caterpillars, and Apanteles colonies emerged from two of the larvae on May 3. The egg and larval stages together required 12 days, at an average temperature of 77° F.

Microplitis varicolor Viereck

Microplitis varicolor Vier. was reared in four cases from larvae of *Cirphis latiuscula* collected from oats and barley. It was not reared from larvae collected from sugar cane. This parasite, which is solitary, was also reared from *Cirphis unipuncta*. Its larva emerges from small caterpillars, about the fourth stage, in the same manner as Meteorus emerges and does not immediately kill the host caterpillar. Only one parasite emerges from each host and spins a small, oval, slightly ribbed, parchmentlike, pale brown cocoon, 4 to 4.5 mm. long by 1 mm. wide.

Meteorus laphygmae Viereck

Meteorus laphygmae Vier. is a common parasite of lepidopterous larvae in the lower Rio Grande Valley. It was reared from the larvae of *Cirphis latiuscula* collected from sugar cane in November, December, March, and April; and from larvae collected from oats or barley in February, March, and April. *Meteorus laphygmae* was reared from 1 per cent of larvae collected from sugar cane, 4 per cent of larvae collected from oats and barley, and 2 per cent of all larvae collected.

The females of *Meteorus laphygmae* oviposit in caterpillars of the first and second stages and the solitary larva of the parasite emerges

from caterpillars of the fourth and fifth stage without killing its host, which usually dies, however, within a few days.

The larva of this parasite spins a transparent, firm, oval cocoon about 2 mm. in diameter and 4.5 to 5 mm. in length, which is attached to the host plant or other object by a thread of silk usually longer than the cocoon. The adult emerges from the cocoon in from 5 to 20 days.

Paniscus sp.

The first larvae of *Cirphis latiuscula* infested with the larvae of Paniscus (fig. 2, D) collected by the writer were collected in sugar-cane fields in April, 1914, 13 infested caterpillars having been taken during that month. Infested caterpillars were also collected in November and December in 1914, and in March and April in 1915, from sugar-cane fields. No Paniscus larvae were found on caterpillars collected from barley or oats. Only 2 per cent of all larvae collected from sugar cane were infested with Paniscus larvae. The writer was unable to rear the adult parasites from any of the caterpillars infested with Paniscus, and it was impossible to determine the species.

Enicospilus purgatus (Say)

Enicospilus purgatus (Say) was reared from *Cirphis latiuscula* larvae collected at Brownsville in only one instance. A caterpillar was taken from corn on December 2, 1913, and from it the larva of Eniscospilus emerged and spun its cocoon about December 15, 1913. The adult parasite emerged from the cocoon on April 25, 1914. The time within the cocoon was about 130 days. This parasite was also reared from *Cirphis unipuncta*.

Euplectrus platyhypenae Howard

The larvae of *Euplectrus platyhypenae* Howard (fig. 2, A) feed externally on the host caterpillar in a cluster. They finally destroy the caterpillar and spin their cocoons in a row under its stretched-out skin. When feeding on *Cirphis latiuscula*, these larvae are greenish yellow in color instead of the darker green which is characteristic of them when feeding on the larvae of *Laphygma frugiperda*.

Euplectrus platyhypenae was reared from larvae of *Cirphis latiuscula* collected in October, November, December, March, and April. It was found to infest only 3 per cent of the larvae from sugar cane, 1 per cent from barley and oats, and 2 per cent of all larvae collected. The adults appear to be long-lived, as reared specimens were kept in the laboratory as long as 5½ months.

In one life-history experiment with *Euplectrus platyhypenae* conducted in the laboratory at Brownsville, eggs of the parasite hatched on a larva of *Cirphis latiuscula* at 5 p. m., April 16, 1914, and the Euplectrus larvae reached full growth and spun cocoons during the afternoon of April 20, 1914. Twenty-one adult parasites emerged from the cocoons between April 27 and 29. The larva stage required 4 days at a mean temperature of 74° F., and the pupa stage about 8 days at a mean temperature of 75° F.

Archytas piliventris Van der Wulp

The tachinid *Archytas piliventris* Van der Wulp is important as a parasite of lepidopterous larvae in the Gulf-coast region of Texas. Its life history was observed in the laboratory at Brownsville in connection with the studies on the parasites of *Laphygma frugiperda*. These flies deposit young larvae on the food plant of the caterpillar, and these larvae catch hold of and crawl onto the host caterpillar as it crawls over them. The parasitic larva soon enters the host and completes its development after the caterpillar changes to the pupal stage. The puparium of the fly is formed inside the pupa of the host a few days after pupation. Only one puparium is formed in each pupa, for the puparium is almost as large as the pupa itself.

Archytas piliventris was reared from the pupae of *Cirphis latiuscula* larvae collected in October, November, December, and April, but it was most numerous in larvae collected in April. This species was reared from 6 per cent of all larvae collected, but infested only 1 per cent of larvae from barley as compared with 7 per cent from sugar cane. Only 44 per cent of the larvae collected from sugar cane pupated, and Archytas adults emerged from 16 per cent of these pupae. Since the 6 per cent destroyed by this species represent those caterpillars that escaped or survived the attacks of other parasites, it should be considered an important parasite of *Cirphis latiuscula*.

Archytas piliventris is diurnal and deposits its larvae on the host plant during the daytime, but in the case of *Cirphis latiuscula* it is not known whether the maggots of the parasite are deposited on the leaves of sugar cane where the caterpillar feeds, or on the dried or fallen leaves or trash where it hides.

A *Cirphis latiuscula* larva pupated the night of April 7–8, 1914, and a puparium of Archytas was formed April 18, from which the fly emerged April 30, or 22 days after the caterpillar pupated. The puparium stage of the fly lasted 12 days at an average temperature of 76° F. In another case, three larvae of *Cirphis latiuscula* pupated between April 16 and 18 and two Archytas adults emerged on May 7, 1914, and one on May 8, or from 19 to 22 days after the caterpillar pupated.

Peleteria robusta Wiedemann

Peleteria robusta Wiedemann (fig. 3, D) was reared only from larvae of *Cirphis latiuscula* collected from sugar cane on one plantation in April, 1914, and it infested 6 per cent of the larvae collected during that month. Only 1 per cent of all larvae collected were infested by this species, which is also a parasite of *Cirphis unipuncta*.

The puparium of Peleteria is formed inside the skin of the full-grown caterpillar, and the skin dries around it, giving to it the characteristic appearance shown in Figure 3, D. The life history of this species was not ascertained in the writer's work at Brownsville.

ARMY-WORM PARASITES NOT REARED FROM CIRPHIS LATIUSCULA CATERPILLARS

Besides the parasites of *Cirphis latiuscula* considered in the preceding paragraphs, there probably were others present at Brownsville which might have been reared if a larger number of caterpillars in the second and third stages had been collected from barley, oats,

and sugar cane. Other species might have been reared also if collections had been made from sorghum hay fields during the summer, or from corn, sorghum, and grasses under conditions such as occurred

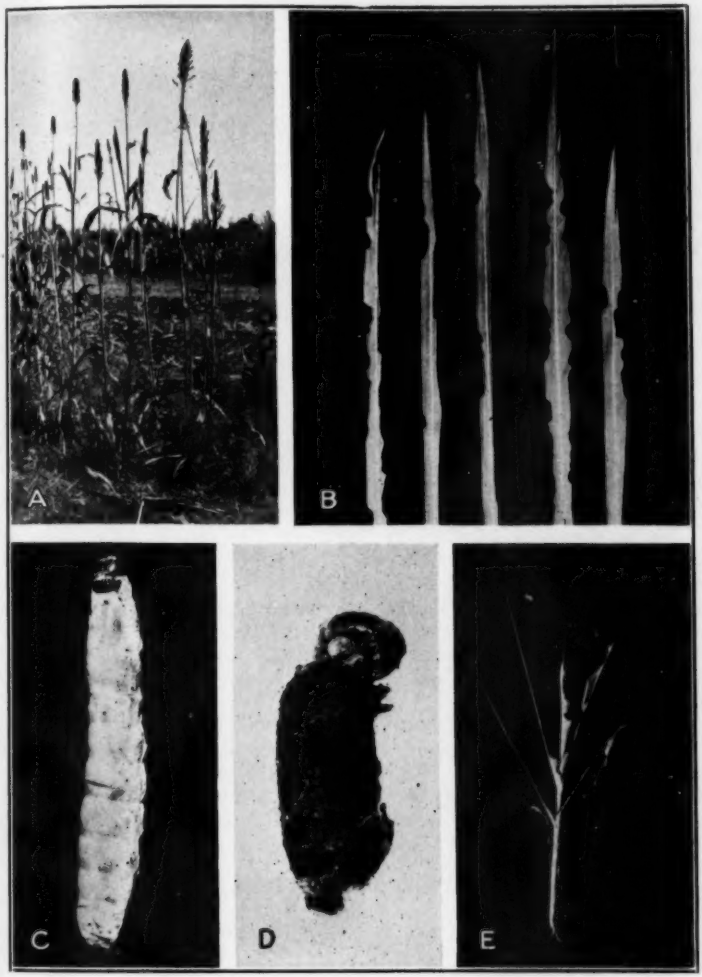

FIG. 3.—A, patch of sorghum defoliated by larvae of *C. latiuscula;* B, leaves of sugar cane injured by larvae of *C. latiuscula*; C, caterpillar of *C. latiuscula* killed by *Beauveria globulifera* (it is covered with the white mycelium of the fungus); D, dried skin of a caterpillar of *C. latiuscula* containing a puparium of *Peleteria robusta*; E, small sugar-cane plant injured by the caterpillars of *C. latiuscula*

at Brownsville in October, 1910, when there was a local army-worm outbreak at that place.

Four species of parasites were reared from caterpillars of *Cirphis unipuncta* collected from oats and barley at Brownsville which were

not reared from the larvae of *Cirphis latiuscula* collected in the same situation. These were *Apanteles marginiventris* Cresson, *Rogas atricornis* Cresson, *Ichneumon brevipennis* Cresson, and *Archytas analis* Fab. These species are mentioned here because they probably would have been reared from *C. latiuscula* if a larger number of caterpillars had been collected.

Beauveria globulifera (Speg.) Picard

Beauveria globulifera is a common parasitic fungus (fig. 3, C) on lepidopterous larvae in the lower Rio Grande Valley. Larvae of *Cirphis latiuscula* infested by this fungus were collected from sugar cane in November and April, and from oats or barley in February, March, and April. Fewer than 1 per cent of all larvae collected were infested.

Larvae attacked by this fungus die stretched out. When dead they are stiff and many for a time look lifelike, but they soon become covered with a white mycelium, and later green spores appear.

SUMMARY

Cirphis latiuscula H. Sch., a moth of the family Noctuidae which is known from the American tropics and from the southern parts of Texas, New Mexico, and Arizona, has been mentioned in the economic literature as injurious to sugar cane and grasses in Porto Rico, but has not been recorded as injurious to crops in the United States, except in a short paper by the writer published in 1915.

This species was studied by the writer at Brownsville, Tex., as opportunity offered, between 1910 and 1917. The larvae are common in the sugar-cane fields during the winter and early spring, and occur on barley or oats during the winter in association with the larvae of *Cirphis unipuncta*. *C. latiuscula* is an army worm similar in habits to the common army worm, *C. unipuncta*, and may be associated with other species in local army-worm outbreaks throughout its range.

The parasites of this species were studied, and many caterpillars were collected and reared to determine the relative importance of the parasites. The most important species were found to be the common parasites of cutworms and army worms, *Apanteles militaris* and *A. rufocoxalis*, and a common tachinid parasite of southern Texas, *Archytas piliventris*.

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THE BIOLOGY OF MACROCENTRUS ANCYLIVORA ROHWER, AN IMPORTANT PARASITE OF THE STRAWBERRY LEAF ROLLER (ANCYLIS COMPTANA FROEHL.)[1]

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INTRODUCTION

During investigations on the biology of the strawberry leaf roller (*Ancylis comptana* Froehl.) in 1920 and 1921, 12 species of parasites were reared. The most abundant and effective was a large yellowish braconid, regarded as new to science, which was later described by Rohwer as *Macrocentrus ancylivora*.[3] Subsequent investigations indicated that in some localities of New Jersey parasitism, frequently attaining 60 per cent, was due entirely to this braconid. In view of its importance, and the fact that it is not known to occur in other sections of the United States where the leaf roller is present, a study of its life history and habits seemed desirable. It was hoped that such a study would throw light on the advisability of introducing it into other sections where its host is destructive.

The preliminary work initiated in the summer of 1920 was continued until 1922, during which time many records of the habits of the parasite in the field, as well as under laboratory conditions, were obtained. A more detailed investigation of its life history was started in the spring of 1923 in the field laboratory at Riverton, N. J. The present paper is, therefore, the result of studies conducted on this parasite for three years.

METHODS OF INVESTIGATION

In order to obtain unparasitized larvae of a known stage on which the parasite might oviposit, strawberry plants were grown in 6-inch pots and inclosed with cylindrical wire-cloth cages in which adults of the leaf roller were confined for oviposition. The stages of the larval host attacked could easily be determined by introducing adult Macrocentrus for short periods into cages containing a known stage of the larva.

To obtain specimens of Macrocentrus, nearly full-grown strawberry leaf rollers were collected in the field and kept in the laboratory until the full-fed parasite larva emerged from the host, spun its cocoon, and pupated. The cocoons containing the Macrocentrus pupae were then placed in tin salve boxes and kept at temperatures ranging from 10° to 15° C., for the purpose of retarding their develop-

[1] Received for publication Oct. 22, 1925; issued June, 1926.

[2] The writer expresses his appreciation to the Department of Zoology of the University of Pennsylvania for the facilities extended him during a part of these investigations, and his obligations to S. A. Rohwer, of the Bureau of Entomology of the United States Department of Agriculture, and to Philip P. Calvert, of the University of Pennsylvania, for many suggestions in the preparation of the manuscript.

[3] ROHWER, S. A. A NEW MACROCENTRUS REARED FROM THE STRAWBERRY LEAF ROLLER (HYMENOPTERA, BRACONIDAE). Proc. Ent. Soc. Wash. 25: 108. 1923.

ment until suitable host material was on hand. At different intervals as needed, the cocoons were taken out of storage and permitted to develop at room temperature (22° to 25° C.). Each lot was timed to issue on a date suitable for oviposition on different stages of the host larva.

On emerging, a male and female Macrocentrus were placed in a glass tube measuring 25 by 90 mm., the open ends of which were

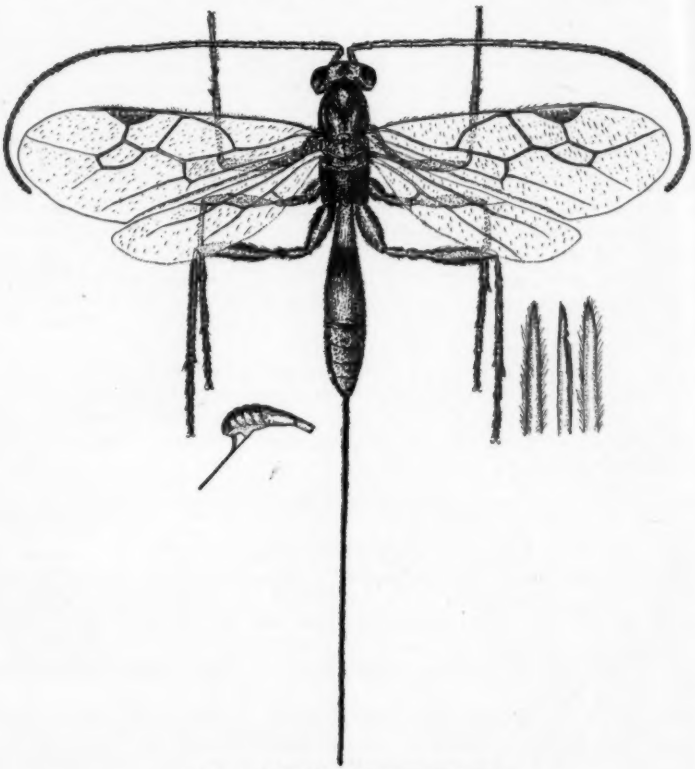

FIG. 1.—Adult of *Macrocentrus ancylivora*

closed with muslin. Macrocentrus when thus confined were fed diluted honey or fruit juices. After mating was known to have occurred, they were placed in wire-cloth cages over plants containing suitable host larvae, for oviposition.

The development of the egg and larval stages of Macrocentrus was traced from numerous dissections of the host made three times a day. In addition, different stages of the embryonic development of the egg and various larval stages were fixed, stained, and mounted for study and photographic record.

THE ADULT

DESCRIPTION

General color pale ferruginous; eyes and ocelli black; antennae brown; base and apex of abdomen of male sometimes marked with brown; sides and venter of thorax and venter of abdomen somewhat more yellowish; legs yellow. Female body about 4.5 mm. in length, ovipositor 5.5 mm., antenna 7.5 mm., and composed of 43 to 47 segments, distal half gradually tapering toward the tip. The wing expanse averages 8.5 mm. The male body averages 4 mm. in length, antenna 6.5 mm. (fig. 1).

COPULATION

The adults usually copulate almost as soon as they emerge, although there are exceptions where copulation does not occur until several hours afterward. The act of coition is brief, lasting only 10 to 15 seconds, the male mounting the female in the usual manner. A female once fertilized usually avoids further attention from the male, but a male may apparently copulate several times with other females. When a male and female are placed together in a vial the male approaches the female, raises his wings at a slight angle, and follows the female about, repeatedly attempting copulation until successful. There is some evidence that the male is attracted by an odor emitted by the female, for when the male is introduced into a glass vial previously occupied by a female, he becomes excited and behaves in the same manner as when in the presence of a female.

LENGTH OF LIFE

The life of the adult as determined from laboratory experiments is brief when confined in vials indoors or in screen cylinder cages outdoors. The females average eight days, the males five days. Individuals which are not fed die sooner.

It is well known that many parasites are more active when exposed to, than when protected from, light. In order to determine whether the life of Macrocentrus could be lengthened by shielding it from direct light, adults of approximately the same age, obtained from material kept under the same conditions of temperature and moisture, were separated into two lots. One group, comprising about 20 individuals, was kept exposed in a well lighted room. The other group, kept in the same room, was protected from direct light by shading with Manila paper. With respect to feeding, temperature, and moisture conditions, both groups received identical treatments. The results of these tests indicated that length of life is not affected by absence of light.

PARTHENOGENESIS

Unfertilized females begin ovipositing a day or two later than those which have been fertilized, but their progeny are males. When these males mate with unfertilized females, the resulting progeny includes individuals of both sexes. Males produced from parthenogenetic females are smaller in size than males produced from fertilized females, and weigh in many instances only about half as much (0.0036 and 0.0054 gm.). Other differences also may be present, especially the physiological reactions in these two forms during the process of histolysis and pupal development. To determine this point, the

rates of respiratory metabolism were measured by the methods described by Jacobs [4] and the present writer.[5] The chief advantage of these methods is the ease with which minute amounts of carbon dioxide given off by a single pupa may be determined. In these experiments the procedure followed was to obtain the daily rates of the carbon-dioxide output of each pupa throughout its development to the adult stage. The results, based on 30 individuals, were averaged and plotted on cross-section paper as shown in Figure 2. These curves reveal some striking differences between the two groups of males. In the first place, it was found that the total carbon-dioxide output during both histolysis and pupal development was the same in males, whether obtained from unfertilized or from fertilized

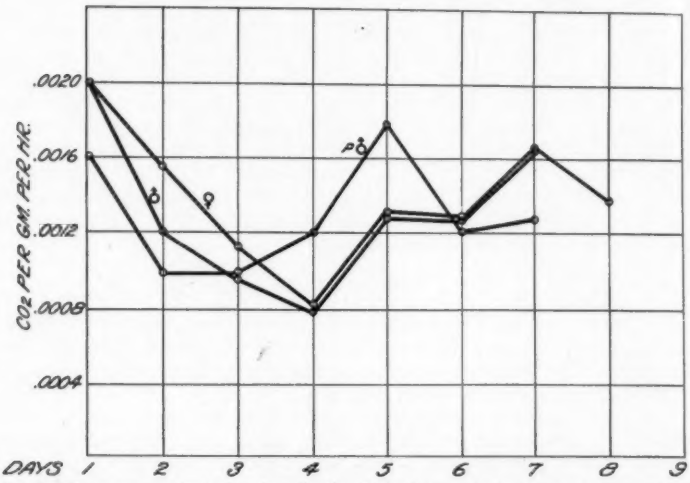

FIG. 2.—The average output of carbon dioxide obtained from single pupae of *Macrocentrus ancylivora* during histolysis and pupal development. P♂ is the curve obtained from males reared from unfertilized females; ♂ is the curve obtained from males reared from fertilized females; and ♀ the curve from female pupae

females (0.0091 and 0.0096 gm. per gram of body weight). However, in the former the rate of carbon-dioxide output during histolysis is less than in the latter (0.0036 and 0.0050 gm. per gram of body weight); and during pupal development it is greater (0.0055 and 0.0043 gm. per gram of body weight).

HABITS OF THE FEMALE MACROCENTRUS LEADING TO OVIPOSITION

A female, on alighting upon a leaf which harbors host larvae, seeks the under surface. As soon as a larva is discovered, the wings of Macrocentrus are raised slightly, the abdomen is lifted almost perpendicularly, the body elevated to the full extent of the legs, and the ovipositor is bent under the abdomen. In this position, and

[4] JACOBS, M. H. A SIMPLE METHOD OF MEASURING THE RATE OF RESPIRATION OF SMALL ORGANISMS. Amer. Nat. 54: 91–96. 1920.

[5] FINK, D. E. METABOLISM DURING EMBRYONIC AND METAMORPHIC DEVELOPMENT OF INSECTS. Jour. Gen. Physiol. 7: 527–543, illus. 1925.

with the antennae probing the surface, every portion of the leaf is inspected. As the ovipositor comes in contact either with the webbed retreats of the host, or with two leaves which are webbed together, several thrusts are made in rapid succession. The ovipositor has not been observed to penetrate the tissues of the leaf, but is thrust into the retreats of the host, whether they are located in a folded or rolled portion of a leaf, or between leaves which are webbed. A host larva receiving a thrust of the ovipositor leaves its shelter in haste and suspends itself from the leaf by a thread.

The wound made in the host by the ovipositor is sometimes visible under the microscope. It may occur on any portion of the body, but in most instances it was found near the caudal end, such as the last three segments of the abdomen. In other instances, however, it has been observed upon the thorax. If the thrust of the ovipositor has been horizontal to the body, the egg may be inserted just under the epidermis. If the thrust has been perpendicular, there is visible only a blackened area, measuring approximately 0.226 mm. in diameter, although the hole itself measures only 0.05 mm. Some females were observed to stop ovipositing only for brief periods to clean the ovipositor and antennae, whereas other females rested for longer periods. One female attempted oviposition continuously for more than 95 minutes, and afterwards rested upon a leaf for the next five hours. Oviposition usually takes place in the morning, only rarely in the afternoon.

Superparasitism is probably explained by the fact that the female seems unable to distinguish between parasitized and unparasitized host larvae. It is quite common to find several Macrocentrus larvae of the first stage, frequently as many as 18 to 24 within a single host, although more than one never survives. Superparasitism was found in larvae collected in the field, as well as in larvae examined from cages in which only one female Macrocentrus was present.

STAGES OF THE HOST ATTACKED

Macrocentrus attacks the host in the larval stage. Experiments have shown that the parasite may oviposit in host larvae that are only a few days old, and in every stage of growth thereafter. When oviposition occurs in first-stage larvae only a small percentage of such larvae are able to continue their growth, and consequently but few parasites develop from such material. It is probable that in other instances the retardation of the growth of young host larvae is due to the wound caused by the ovipositor of Macrocentrus. The retardation of growth of the host likewise retards the development of the parasite egg and the growth of the first larval stage. This retardation will be discussed later.

THE EGG

At the time of oviposition, the egg deposited by fertilized females averages 0.08 by 0.012 mm. The caudal end is rounded, and the cephalic end narrows to either a straight or curved blunt point. The egg is distinctly hyaline, showing only the outline of the chorion. After staining, the nucleus is readily detected near the cephalic end.

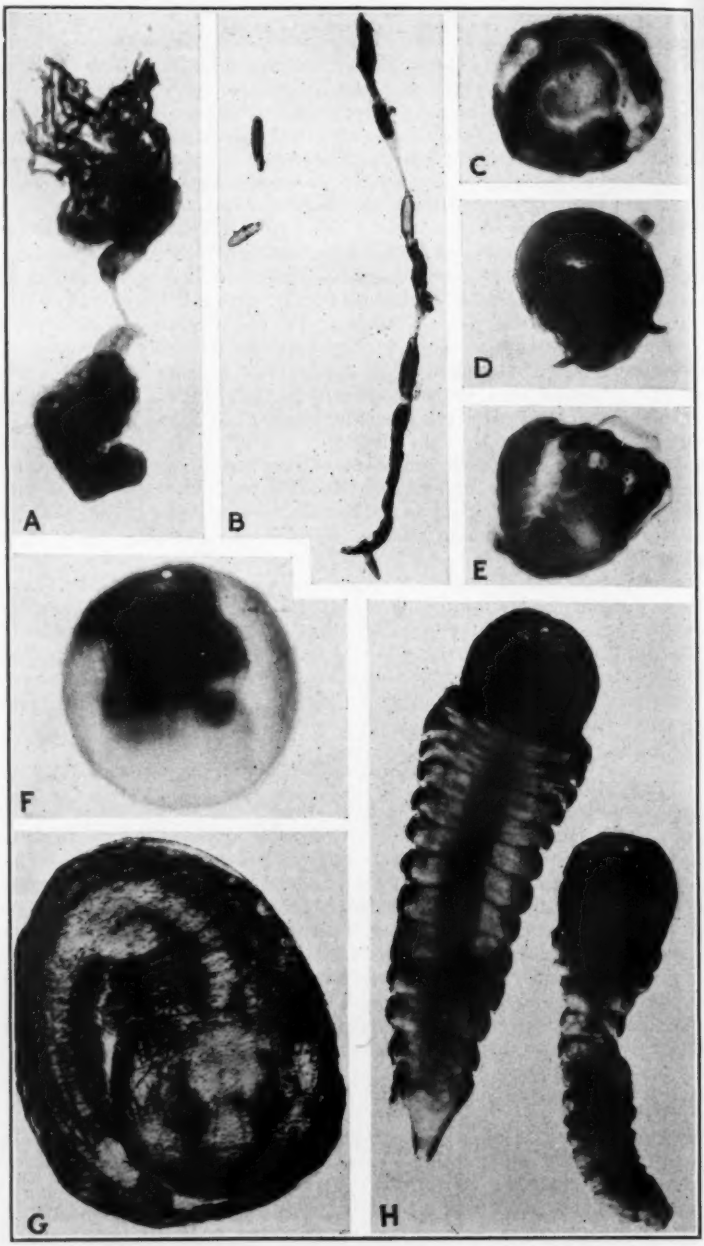

FIG. 3.—*Macrocentrus ancylivora:* A, two ovaries, ×67; B, ovarian tube and single eggs, ×106; C to G, embryonic development of the egg, ×106; H, first-stage larvae, the smaller of the two showing where the body was nipped by its rival, ×67

The number of eggs which a female contains varies with the individual. To determine this point it was necessary to dissect. The number of ovarian tubes varies with individuals; in some females the number in each ovary was 24, in some 28, and in others 32. Each ovarian tube contained from 8 to 12 eggs; 4 to 6 fully developed eggs were apparently ready to be deposited, and the remainder were in various stages of development. The minimum number of eggs in all stages of development contained in both ovaries was 384, and the maximum number was 768.

The ovarian tubes of this species are evidently of the type in which eggs and nutritive chambers of the developing eggs alternate. Almost half of the eggs of a day-old fertilized female are about fully developed. This is further shown by the large number of nutritive cells that have disappeared, forming narrow constrictions between the developed eggs.

CHANGE IN APPEARANCE OF THE EGG

The most striking feature observed while studying the egg was the change in shape and size after oviposition. When first deposited, the egg is long and narrow, but gradually it changes shape and becomes more and more spherical in outline. As it rounds out it gradually increases in diameter, this increase keeping pace with the development of the embryo. The average measurements of the egg when first deposited were 0.08 by 0.012 mm., and the average size of the egg when ready to hatch was 0.522 by 0.486 mm.

INCUBATION

The incubation period could be determined only approximately, although a large series of the host larvae of a known date of parasitism were dissected. The incubation period ranged from 3 to 13 days. The rapidity of the development of the embryo seemed to depend upon the stage of the host parasitized, it being greater when oviposition had taken place in the last two stages of the host. First or second-stage larvae in which the Macrocentrus had oviposited a week previous to dissection contained only imperfectly developed eggs, whereas host larvae of the third stage into which eggs had been deposited at the same time often contained fully developed eggs. It is believed that the hatching of eggs deposited in young larvae is retarded until the larvae have grown to sufficient size to accommodate the parasite. In Table I are summarized the records showing what effect each stage of the host larvae exerts on the development of the parasite egg.

TABLE I.—*Incubation of Macrocentrus ancylivora*

Date	Stage of host larva in which oviposition took place				Date of hatching	Incubation period	Mean temperature
	First	Second	Third	Fourth			
1923						*Days*	°*C.*
July 1	*				July 14	13	25
2	*				15	13	25.5
2		*			14	12	25
2		*			15	13	25.5
8			*		12	4	25.5
10			*		16	6	27
15			*		18	3	27
20				*	23	3	22.5
21				*	24	3	21

THE LARVA

FIRST STAGE

While the larva is still surrounded by the transparent chorion of the egg its movements very often can be observed with the aid of a binocular microscope. By straightening out its folded abdomen the larva causes the egg membrane to stretch, thus bringing it within reach of its sharp pointed mandibles which slit the anterior end, and the larva emerges from the egg membrane. On hatching, the body of the larva averages 0.63 mm. in length, the head 0.25 mm. in width, and the mandibles 0.1 mm. in length. The body (exclusive of the head) has 13 segments, is widest at the first segment, each succeeding segment narrowing gradually toward the anal extremity. The anal segment has three curved hyaline prolongations, which probably aid the larva in locomotion or are useful for attachment. These prolongations are absorbed at about the time the larva molts.

The first-stage larva is further characterized by a very large chitinized head, light brown in color, bearing a pair of strongly chitinized mandibles that are sicklelike in form and acute at the apex. Broadly separated at the base, the mandibles are capable of wide movement and can get a tenacious hold on objects within the host, such as tissues or other larvae hatched within the same host. The writer placed the fine point of a dissecting needle within reach of these mandibles, and the larva closed its mandibles on it with such a grip and clung so fast that it was possible to lift it out of the medium.

Just above the base of the mandibles and toward the middle of the anterior portion of the head a pair of modified maxillae, together with the labrum, may be distinguished, functioning like a suctorial organ. The parasitic larva feeds almost entirely upon predigested food, consisting largely of lymph, body fluids, and fat globules.

The alimentary canal is readily visible through the integument. Starting anteriorly with the short narrow oesophagus, it gradually widens into the long mid intestine and extends to the anal segment, where it narrows slightly; internally there is no communication with the adjoining short proctodaeum. The hind gut (proctodaeum), which is round and sacklike, is located in the caudal segment and constricts slightly toward the oval anus, which in this stage is located on the dorsal surface anterior to the anal prolongations mentioned above. The posterior portion of the proctodaeum becomes more or less roundly mammilate or nipple-shaped. The cells composing this

part of the hind gut are elongated toward the nipple end. As the larva grows this portion of the hind gut protrudes slightly above the anus. With each succeeding stage of growth of the larva, the extruded portion becomes larger, forming a hollow cone above the anal opening. This structure closely resembles the anal vesicle described by Weissenberg [6] in his careful studies on the structure of the anal vesicle in *Apanteles glomeratus* L. and an undetermined species of Macrocentrus; Weissenberg concludes that in the latter species a true anal vesicle is not formed.

It is possible to show experimentally that in *Macrocentrus ancylivora* the protruded cells are an outgrowth of the posterior portion of the hind gut. When larvae of the first, second, and third stages are immersed in distilled water, tap water, or weak saline solutions of 0.1 to 0.4 per cent, the absorption of water develops osmotic pressure, causing a swelling of the body and alimentary tract.

The pressure thus created is exerted against the weakest portion of the body, in this instance against the extruded portion of the hind gut. Ultimately the part that forms a hollow cone over the

FIG. 4.—First-stage larva of *Macrocentrus ancylivora*, full grown, showing the alimentary tract, a suggestion of anal vesicle at A, and the anal appendages at B

anus breaks off, followed by an extrusion of other portions of the proctodaeum, consisting mostly of a broken mass of cells.

A closed tracheal system is present. Two main tracheal trunks appear on the lateral sides of the body and terminate in the head and anal segments as finely divided, branched tubes. From each of these lateral main trunks a pair of finely multibranched tracheae extends to each body segment. The divisions of these branched tracheae seem to ramify the entire integument. Tracheae are present in every larval stage of Macrocentrus, the branching becoming extremely complex and extending to all parts of the body of the more matured larvae. While the larva exists as an endoparasite, stigmata or spiracles are absent; spiracles appear just before the parasite emerges from the host.

The growth and development of the first larval stage are apparently determined by the condition and growth of the host. The first-stage larva of Macrocentrus attains considerable growth before molting; measurements made just before the larva molts gave an average of 2.3 mm. in length and 0.32 mm. in width.

Owing to the strong chitinization of the head, the cast skin of this stage is very readily found when the host is dissected. In other molts, however, the cast skins are very often broken into fragments and are difficult to recover (fig. 4).

[6] WEISSENBERG, R. ZUR BIOLOGIE UND MORPHOLOGIE ENDOPARASITISCH LEBENDER HYMENOPTERENLARVEN (BRACONIDEN UND ICHNEUMONIDEN). Sitzber. Gesell. Naturf. Freunde Berlin 1909: 1–28, illus. 1909.

SECOND STAGE

After the first molt the appearance of the larva has changed considerably. The head is narrow and not chitinized. The mandibles, distinguished with difficulty, are somewhat broad at the base, point inward at the tips, and are but slightly chitinized. With growth and feeding, the body becomes more uniform and the segments more distinct. The fleshy hornlike protuberances of the anal segment, characteristic of the first stage, are absent. The larva averages 2.77 mm. by 0.86 mm. This stage lasts from two to three days (fig. 5).

FIG. 5.—Second-stage larva of *Macrocentrus ancylivora:* Anal vesicle at A; mouth at B

THIRD STAGE

After the larva molts a second time, the mandibles measure 0.15 mm. in length and are slightly chitinized. It has been previously stated that these molts are difficult to find. In some dissections the larvae were in the process of molting. In one instance part of the cast skin was adhering to the anal segment, the head and first several segments of the body being still incased in another portion of the molted skin. In this individual the old skin evidently had split transversely, and the larva freed itself by means of body contortions.

After extensive feeding, the larva of the third stage becomes gorged with liquid food. The skin, distended by the full intestine, gives the larva an elongated sacklike appearance. Movements of the larva are confined largely to the small head and upper portions

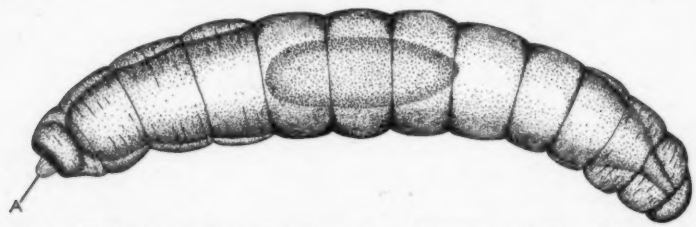

FIG. 6.—Third-stage larva of *Macrocentrus ancylivora*, showing digestive tract; anal vesicle at A

of the body. With additional growth the parasitic larva occupies nearly two-thirds of the body cavity of its host and causes more or less flattening of the alimentary canal of the latter. The third-stage parasite larva before molting measures 3.74 mm. in length by 0.81 mm. in width (fig. 6).

FOURTH STAGE

The habits of the parasite larva change with the last molt. It no longer continues its existence as an internal parasite within the host,

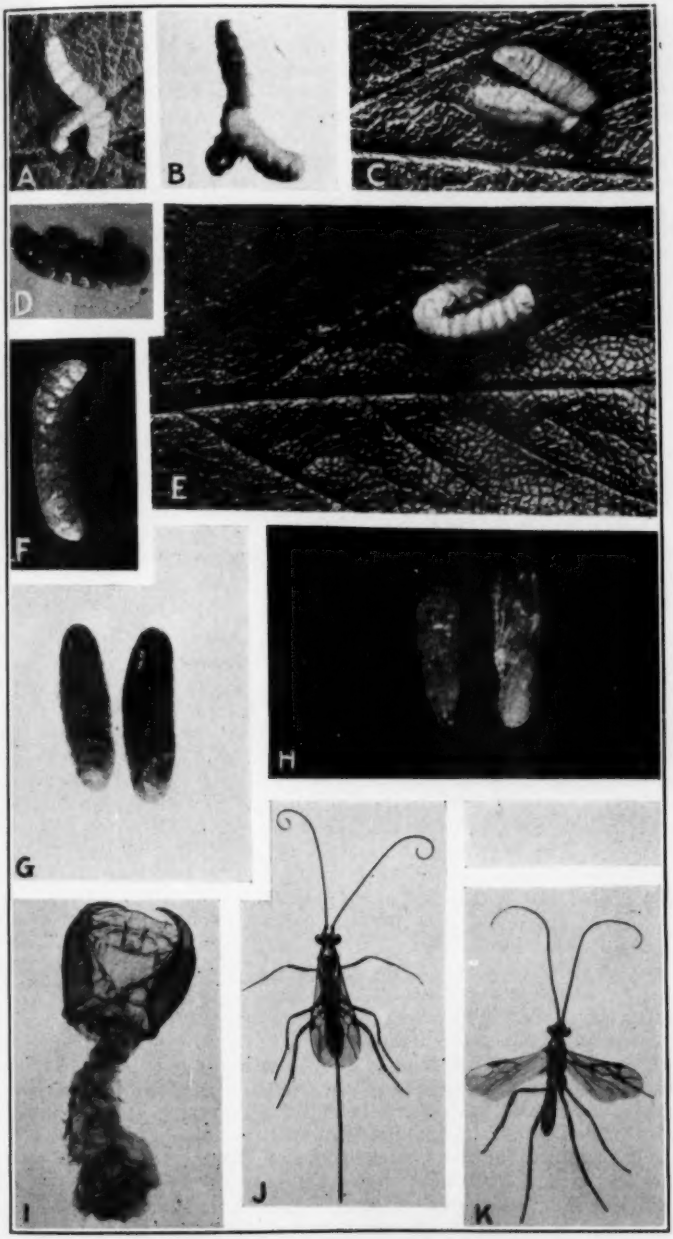

FIG. 7.—*Macrocentrus ancylivora:* A and B, larva emerging from the host, ×6.5; C to E, larva at various intervals feeding upon the host from the exterior, ×6.5; F, full-grown larva ready to construct its cocoon, ×6.5; G, cocoons, 6.5; H, male and female pupae, ×6.5; I, the molted skin and head of the first-stage larva, ×77; J and K, female and male

but emerges from the body cavity to complete its cycle outside. The process of emergence is as follows: First the caudal end of the parasite larva appears through an opening in the body of the host and the larva gradually works its way out by body contortions which take from one-half to three-quarters of an hour. After emergence the parasitic larva lies parallel to its former host, and with its head embedded in the opening continues to feed further until nothing remains of the host except the chitinized head and shriveled skin (fig. 7). The full-grown larva averages 4.5 by 1.5 mm., and is yellowish white to gray in color. The body is uniform in width, and the head is larger and more prominent than in the previous stage (fig. 8).

FIG. 8.—Full-grown larva of *Macrocentrus ancylivora*, fourth stage

THE COCOON

After disposing of its host, the full-grown larva spins its cocoon. Since the parasite larva is incapable of locomotion, the cocoon is spun in a folded or webbed part of the leaf where it has emerged from the host. Three layers of silk form the envelope of the cocoon. First, strands of silk loosely spun by the larva on the leaf serve as an attachment and outline; the second layer, brownish and very closely woven, forms the oval cocoon. The anterior end of this layer is made exceptionally thick by the addition of several separate circular layers, which look very much like little caps. A third layer of a lighter silk, also very closely woven but much more delicate in structure than any of the preceding ones, finally incloses the parasite pupa. The time consumed in the construction of the cocoon varies from 24 to 36 hours. Its measurements when completed average 4.5 by 1.5 mm. (fig. 7, G).

THE PREPUPA

After the cocoon is formed the larva prepares for pupation. There follows a quiescent period lasting from 24 to 36 hours, during which all the accumulated material of the intestine is expelled. As transformation to pupa takes place, the larval skin, commencing at the cephalic end, splits and is gradually rolled posteriorly, revealing the newly formed pupa. This cast skin and the expelled intestinal contents may remain at the posterior end of the cocoon, but the movements occurring during the process of pupation may cause the excreted adhesive mass to lie somewhat mid dorsally. In the pupa of the male, a part of this mass adheres to the apices of the antennae; in the pupa of the female, to the ovipositor and the antennae.

THE PUPA

When first formed the pupa is white, with reddish eyes. With later development its color changes gradually to a yellowish brown. The pupa of the female averages 4.5 by 1.3 mm. in size, that of the male 4.2 by 1.2 mm. (fig. 9).

When the adults are ready to emerge, they make a slit at the anterior end of the cocoon, nearly severing the cap from the main part. The adults escape readily without any part of the excreted material of the larvae adhering either to the ovipositor or to the antennae. Pupation lasts from 8 to 10 days, but it may be extended if climatic conditions are not favorable, or very low temperatures prevail. If newly formed pupae are subjected to temperatures as low as 10° to 15° C., the rate of development may be greatly retarded. At these temperatures 5 to 6 days were equivalent to 1 day's development under normal temperature (22° to 25° C.). That development does take place at low temperatures, however, was shown by the fact that adults issued from material while it was held in storage for two months.

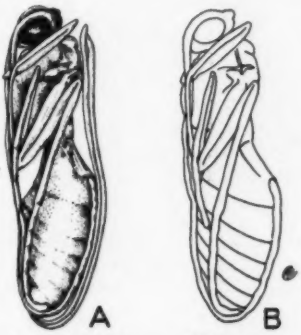

FIG. 9.—A, female pupa of *Macrocentrus ancylivora*; B, male pupa

LENGTH OF LIFE CYCLE

The length of the life cycle, determined from many rearing experiments, shows a maximum of 38 days and a minimum of 20 days. The maximum or minimum period in all stages of development does not usually occur in the life cycle of an individual. The maximum or minimum development may occur in a single stage, or at most in two. The two stages of the parasite most likely to be retarded are the egg and the first-stage larva. The reasons for such retardation have already been stated. Table II, which shows the minimum and maximum periods required for development of the life cycle of Macrocentrus, indicates that the egg, first larval stage, and pupal period are the stages most likely to be retarded or hastened.

TABLE II.—*Minimum and maximum number of days required for the development of each stage in the life history of Macrocentrus ancylivora*

	Egg	Larval stages				Prepupa	Pupa	Total	Temperature
		First	Second	Third	Fourth				
									° *C.*
Minimum	3	3	2	2	1	1	8	20	22-25
Maximum	13	8	2	2	1	1½	10	37½	22-25

NUMBER OF GENERATIONS

The active period of the parasite agrees with that of the host in that every generation of the latter is parasitized. In the vicinity of Riverton, N. J., there are three generations of the host. The most abundant generation of the parasite is the first. This also agrees with the abundance of its host, which is at a maximum in the first generation.

HIBERNATION

Hibernation of Macrocentrus takes place as a mature first-stage larva within the body of the full-grown leaf roller. Dissections of the hibernating host made repeatedly during fall and winter yielded only first-stage parasite larvae. In some instances the dissections revealed also matured eggs of the parasite, but these were dead, and it is known that such eggs are killed by the first-stage larva before hibernation.

SUMMARY

Macrocentrus ancylivora, first reared from the strawberry leaf roller (*Ancylis comptana*), in the year 1920 in the vicinity of Riverton, N. J., was found to parasitize from 50 to 60 per cent of the host larvae.

All larval stages of the host are attacked by Macrocentrus. When the third and fourth stages are parasitized, the length of time required for the development of the parasite is less than when younger stages of the host are attacked.

From dissections of the ovaries it was found that the number of eggs which a female contains ranges from 384 to 768. Incubation of the egg requires from 3 to 13 days; the first larval stage requires from 3 to 8 days for development; the second and third larval stages each requires 2 days; and the fourth larval stage 1 day. The prepupal stage lasts from 24 to 36 hours, and pupation lasts from 8 to 10 days; the adult lives from 5 to 8 days.

There are three generations of this parasite in New Jersey annually, the first being the most abundant.

Macrocentrus ancylivora passes the winter as a mature first-stage larva within the full-grown hibernating host larva of the strawberry leaf roller.

THE COMPARATIVE SUSCEPTIBILITY OF SWEET-POTATO VARIETIES TO BLACK ROT[1]

By L. L. HARTER, *Pathologist*, and J. L. WEIMER and J. I. LAURITZEN, *Associate Pathologists, Office of Vegetable and Forage Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The black rot of sweet potatoes (*Ipomoea batatas* Poir.), caused by *Ceratostomella fimbriata* (E. & H.) Elliott, is one of the most destructive and widely distributed diseases of the crop in the United States. It has been reported from the West Indies, New Zealand, Australia, and Japan, and sweet potatoes brought from the Azores to the United States by immigrants were found to be affected with the disease.

Black rot occurs in the seed bed and in the field, but the greatest loss probably occurs in storage. If infested soil is used in the seed bed, or if diseased potatoes are bedded, the sprouts may become diseased and carry the organism to the field where it develops during the summer with the growing crop. Although only a relatively few, diseased potatoes may be observed when the crop is dug, the causal fungus is likely to be present. In storage it develops and spreads so rapidly that a considerable percentage of the crop may be destroyed in a few weeks.

Heavy losses have given rise to frequent requests from farmers for resistant or nonsusceptible varieties. In the absence of any experimental data, a series of experiments was conducted which had for its purpose a comparative study of the relative susceptibility of a number of the best known and more common varieties of the swete potato grown in the United States.

These varieties, 21 in all, obtained for the most part from the Office of Horticulture, United States Department of Agriculture, were as follows: Yellow Strassburg, Nancy Hall, Porto Rico, Southern Queen, Yellow Jersey, Dahomey, White Yam, General Grant Vineless, Pierson, Pumpkin, Red Brazil, Creola, Georgia, Haiti, Yellow Belmont, Red Jersey, Big Stem Jersey, Gold Skin, Dooley, Key West, and Triumph. All these varieties were tested over a period of four years, except Haiti, Gold Skin and Dooley, which were grown for three years; White Yam, for two years; and Creola and Red Jersey for one year.

PLAN OF EXPERIMENTS

One acre of ground on the Arlington Experiment Farm, near Rosslyn, Va., was selected for the experiments. The soil was in a good state of cultivation and fairly well suited for growing sweet potatoes. Various crops, but not sweet potatoes, had been grown on the land previous to the beginning of these experiments. Infection by the black-rot organism was accomplished by artificial means.

The crop was handled, as far as possible, in accordance with commercial practice, variations being introduced where necessary to meet the requirements of the experiments. Healthy seed potatoes were bedded in the usual way in a manure-heated bed. As soon as they were bedded, and just previous to covering with a mixture of soil and sand, they were sprayed with a spore suspension made from a pure culture of the black-rot organism.

[1] Received for publication Nov. 16, 1925; issued June, 1926.

When the plants were set in the field, the total number of plants was noted, and the black-rotted plants were counted. While it was not always easy to tell by macroscopic examination whether a plant was diseased, some data of a general nature were obtained as to the percentage of black rot in the hotbed. The roots of both the healthy and diseased plants were then assembled, dipped into a suspension of spores of the black-rot organism (except the first year) and planted. The field was laid out in three sections, and two rows of each variety were planted in each section.

Throughout the growing season careful counts were made of the numbers of dead and living plants. Those plants which had died were pulled out and examined. The last two seasons all the plants of one row of each variety in one section, whether dead or alive, were pulled up about one month after planting and examined. This procedure gave very reliable information as to the actual percentage of infected plants in the field. Plants which survived the first month after planting usually lived through the season and produced a crop, although in many cases the roots, and particularly the underground part of the main stem, were actually infected.

When the plants were dug, the total number of sweet potatoes and the number of those infected were recorded. Black rot frequently develops to an alarming extent in storage, even though there is little evidence of its presence when the sweet potatoes are dug. In view of this fact, during the last two years one bushel of each variety was gathered promiscuously from the field and stored at a fairly constant temperature and humidity. The temperature varied from 25–27° C., which is near the optimum for the development of the organism on the roots in storage. In the absence of accurate data as to the optimum relative humidity for this organism, a relative humidity of 84 was employed, which gave very satisfactory results.

RESULTS OF SEED-BED TESTS

The percentages of plants of the different varieties of sweet potatoes infected by the black-rot fungus in the seed bed for the four years 1921 to 1924, inclusive, are given in Table I.

TABLE I.—*Percentage of black-rotted plants of the different sweet-potato varieties affected at time of planting (four-year test)*

Variety	1921	1922	1923	1924
Big Stem Jersey	27.9	8.2	29.4	36.7
Creola				13.0
Dahomey	16.2	6.8	0.0	20.6
Dooley		10.8	0.6	52.5
Florida	41.1			
General Grant Vineless	50.8	6.0	2.9	10.6
Georgia	56.0	32.0	3.3	29.8
Gold Skin	16.7	29.8		32.3
Haiti		3.0	.3	15.7
Key West	60.3	15.0	0.0	31.5
Nancy Hall	54.6	23.0	1.0	16.8
Pierson	28.8	9.4	0.6	11.8
Porto Rico	60.0	34.0	0.0	74.6
Pumpkin	68.1	15.0	0.0	33.0
Red Brazil	37.1	37.0	0.6	9.3
Red Jersey				28.8
Southern Queen	40.0	8.7	1.0	12.6
Triumph	10.3	3.0	9.63	20.7
White Yam			0.0	22.6
Yellow Belmont	42.3	23.0	0.3	20.6
Yellow Jersey	20.0	15.0	2.0	66.6
Yellow Strassburg	6.7	2.0	0.0	13.8

The results shown in Table I are extremely variable. When the sweet potatoes were bedded they were watered with a suspension of *Ceratostomella fimbriata* spores before they were covered with the sand or soil. This rather severe treatment was applied because it was thought that if any varieties remained free of infection under such conditions they must possess some resistance. The results show that all the varieties become infected in varying degrees. Some of the varieties (Yellow Jersey, Pierson, Dooley) showed a relatively low percentage of infection one year and a high percentage another. Some of the varieties which remained comparatively free of infection under one set of conditions were especially susceptible under another. In view of this fact it seems more logical to regard such varieties as having merely escaped infection rather than as exhibiting resistance.

The percentage of infection the first and fourth years was higher than that of the second and third (Table I). These results may be explained, in part at least, by the fact that infection of sweet potatoes by the black-rot organism may be conspicuously influenced by conditions of the soil such as moisture and temperature. The amount of moisture was not entirely controlled, and the temperature not at all after the heat from the stable manure was exhausted. In 1923, when the lowest percentage of infection in general was recorded, the soil of the hotbed was kept relatively dry. However, in 1924, in order to provide what was thought to be more favorable conditions for infection, the bed was watered frequently, and the percentage of infection was perceptibly higher.

RESULTS OF FIELD TESTS

When sufficient time had elapsed after planting for the plants to become established, usually about one week to 10 days, they were counted and the dead plants were removed and examined. The total number of counts made during the season depended somewhat upon the rate of growth of the plants. Counts were made every 10 days to two weeks until the vines had covered the rows so that it was difficult to distinguish the hills. In 1921 and 1922 four counts were made, in 1923 two, and in 1924 three.

No sweeping generalizations can be drawn from these counts. In 1921 the percentage of infection as shown by field counts was low as compared with 1924, a season which was extremely wet about planting time and in general favorable for infection. Furthermore, 1924 was the fourth year and 1921 the first, the plants in the last year having therefore been subjected to the cumulative infestation of the soil of the three previous seasons. The one significant conclusion to be deducted from these data is that none of the varieties employed was immune.

It is generally agreed that plants badly infected with black rot when set out usually die. If they are only slightly infected they may grow normally and produce a fair crop without exhibiting any aboveground symptoms. In order to obtain an accurate knowledge of the percentage of infection in the field, all the plants of one row of each variety in one of the three sections of the field were pulled and carefully examined. These examinations and counts were made

approximately four weeks after the plants were set in the field (Table II). No counts of this kind were made the first year (1921).

TABLE II.—*Percentage of plants of the different varieties of sweet potatoes found infected after growing in the field for four weeks*

Variety	1922 (June 30)	1923 (July 11)	1924 (July 17)	Average
Big Stem Jersey	100	49	100	83
Creola			94.5	94.5
Dahomey	100	52.7	93.3	82
Dooley	100	42.5	98	80.1
General Grant Vineless	94.6	30.8	92.1	72.5
Georgia	100	91.8	97.4	96.4
Gold Skin	100		100	100
Haiti	97.1	38.7	100	78.6
Key West	91.6	14	93.7	66.4
Nancy Hall	100	79	100	93
Pierson	100	37.7	97.5	78.4
Porto Rico	100	33.3	100	77.7
Pumpkin	100	42.8	94.2	79
Red Brazil	100	10.9	97.6	69.5
Red Jersey			100	100
Southern Queen	100	68	95.7	87.9
Triumph	100	37.5	83.8	73.7
White Yam		34.3	96	65.1
Yellow Belmont	100	45.9	100	81.9
Yellow Jersey	99.1	50	100	83
Yellow Strassburg	100	10.7	94.2	68.3

Practically every variety showed a high percentage of infection (Table II). Although the percentage of infection of a particular variety was low one season, it might be high the next, as illustrated by Yellow Strassburg with a 100, 10.7, and 94.2 per cent infection in 1922, 1923, and 1924, respectively. Similar results were obtained with Red Brazil. The Porto Rico, a variety which has exhibited marked susceptibility at times, gave 100 per cent infected plants in 1922 and 1924, and only 33.3 per cent in 1923. The conclusion to be drawn from these data is that all the varieties are susceptible and probably to a more or less uniform degree. The variations exhibited in the amount of infection in the different years are probably due largely to certain favorable or unfavorable conditions.

RESULTS AT DIGGING TIME

Since such a high percentage of the plants was infected in the field, one would naturally expect that most of the sweet potatoes would be diseased when dug. Such, however, was not the case, the percentage of infected potatoes being relatively small. Undoubtedly there were many infections which were not visible but which developed later and served as sources of infection for other sweet potatoes in the storage house. It is evident from the results of the counts that black rot is usually inconspicuous at harvest time, but that it can produce extensive damage in the storage house if present in the crop even to a very limited extent. A considerable amount of evidence for this was obtained by making careful counts of the total number of sweet potatoes of each variety at digging time (1921 excepted) and of the number with visible black-rot lesions. Some of these sweet potatoes were then stored for about six weeks and notes taken of the number of black-rot spots. In 1921 the total number of sweet potatoes was not obtained when dug. However, an examina-

tion that year for black rot gave a total of only 13 diseased potatoes in the entire field, distributed among 8 varieties. In 1922–1924, counts were made of the total number of sweet potatoes and the number of those affected with black rot. The results are shown in Table III.

TABLE III.—*Total number of sweet potatoes and black-rotted sweet potatoes of each variety in the field at time of digging, for the years 1922 to 1924, inclusive*

Variety	1922			1923			1924		
	Total	Number diseased	Percentage diseased	Total	Number diseased	Percentage diseased	Total	Number diseased	Percentage diseased
Big Stem Jersey	436	0	0	766	11	1.4	275	10	3.6
Creola							184	2	1.1
Dahomey	198	0	0	601	4	.66	83	1	1.2
Dooley	305	3	.98	237	8	3.37	82	3	3.6
General Grant Vineless	83	0	0	587	4	.68	143	3	2.0
Georgia	379	3	.79	581	28	4.8	228	10	4.3
Gold Skin	390	0	0				285	4	1.4
Haiti	194	0	0	641	8	1.2	114	1	0.87
Key West				691	0	0	151	2	1.3
Nancy Hall	211	0	0	1,099	50	4.5	265	15	5.6
Pierson	303	0	0	603	12	1.9	201	8	3.9
Porto Rico	256	3	1.17	736	9	1.22	74	13	17.6
Pumpkin	306	0	0	376	13	3.4	209	5	2.4
Red Brazil	209	0	0	693	4	.577	281	5	1.7
Red Jersey							181	3	1.6
Southern Queen	481	0	0	794	10	1.26	184	10	5.4
Triumph	322	0	0	782	17	2.3	343	7	2.0
White Yam				727	5	.68	163	7	4.2
Yellow Belmont	182	0	0	829	34	4.1	142	7	4.9
Yellow Jersey	823	1	.12	2,238	43	1.92	89	3	3.3
Yellow Strassburg	190	0	0	514	0	0	137	2	1.45

Table III shows that with one or two exceptions the amount of black-rot infection at digging time is relatively low for all the varieties In 1924, 17.6, 5.6, and 4.9 per cent, respectively, of the Porto Rico, Nancy Hall, and Yellow Belmont varieties were infected; and in 1923, 4.8, 4.5, and 4.1 per cent, respectively, of the Georgia, Nancy Hall, and Yellow Belmont. The results of these investigations indicate a gradual increase in soil infestation. Although there were some black-rot sweet potatoes at harvest time, data were not obtained in 1921 from which percentage calculations could be made. The data of the three succeeding years indicate that continuous cropping of the same soil brings about an increase in amount of infection. In 1922 there were 10 black-rot sweet potatoes out of a total of 5,268 distributed among four varieties (Yellow Jersey, Porto Rico, Dooley, Georgia), with an infection of 0.19 per cent. The percentage increased to 1.93 in 1923, there being only two varieties (Yellow Strassburg, Key West) of which none of the potatoes were infected.

Judging from the results of these experiments, the amount of black rot on the potatoes at harvest might seem to be of no great importance. The loss in the field under average conditions is ordinarily not more than 1 to 4 per cent. If, on the other hand, one bears in mind the losses in storage as the result of field infections, a clean crop must be regarded as of the greatest importance. The losses which resulted from storing sweet potatoes from a field where black rot occurred will be shown by the data to be presented below.

RESULTS IN STORAGE

The potatoes used in the storage tests were picked up promiscuously in the field after counts were made of the total number of sweet potatoes and of those that were black-rotted. One bushel of each variety, including all sizes, was placed in open crates and stored the day they were dug at a temperature of about 25° C. and a relative humidity of from 84 to 86 per cent. They were then left undisturbed for six to seven weeks. An electric fan blowing over a pan of water in the room was run continuously at a speed sufficient to keep the air in motion but not to create a draught. At the end of the storage period the sweet potatoes were removed and thoroughly cleaned by washing in water. A count was then made (1) of the total number of sweet potatoes, (2) of the number affected with black rot, and (3) of the number of black-rot lesions on each potato. When the data were taken there was considerable difference in the size of the various lesions on the same sweet potato, as well as on those of the different varieties. For example, the lesions on the Georgia variety were small and inconspicuous, while those on White Yam, Yellow Strassburg, Porto Rico, and Nancy Hall were large, in some cases attaining a diameter of from 2.5 to 3 inches. The spots on the Porto Rico were large, conspicuous, and dark in color. Those of the Nancy Hall, besides being large and dark, were conspicuously zonate.

Table IV shows that the number of sweet potatoes in each lot (bushel) varied from 38 (Red Brazil) to 171 (Georgia). The number of lesions per potato varied with the size.

TABLE IV.—*The total number of sweet potatoes, the percentage infected with black rot, the number of lesions per diseased potato, and the average number of lesions per potato, for all varieties, for 1923 and 1924*

Variety	1923					1924				
	Total number of sweet potatoes	Total number of diseased sweet potatoes	Percentage of infection	Total number of black-rot lesions	Average number of lesions per potato	Total number of sweet potatoes	Total number of diseased sweet potatoes	Percentage of infection	Total number of black-rot lesions	Average number of lesions per potato
Big Stem Jersey	73	7	9.5	9	1.3	110	26	23.6	36	1.4
Creola						115	21	18.3	26	1.2
Dahomey	83	24	28.9	34	1.4	53	40	75.4	115	2.9
Dooley	75	46	61.3	171	3.7	43	10	23.2	15	1.5
General Grant Vineless	61	2	3.3	2	1	105	46	43.8	85	1.8
Georgia	73	46	63	135	2.9	171	92	53.8	171	1.9
Gold Skin						104	36	34.6	53	1.5
Haiti	59	26	44	45	1.7	103	83	80.5	377	4.5
Key West	56	12	21.4	20	1.7	71	54	76	144	2.7
Nancy Hall	104	32	30.7	46	1.4	76	57	75	149	2.6
Pierson	42	5	11.9	6	1.2	128	59	46.1	106	1.8
Porto Rico	86	31	36	49	1.6	54	41	75.9	123	3
Pumpkin	78	57	73	153	2.7	108	80	74	246	3.1
Red Brazil	38	31	81.5	122	3.9	90	49	54.4	119	2.4
Red Jersey						118	8	6.8	9	1.1
Southern Queen	61	4	6.6	4	1	92	37	43.2	73	2
Triumph	47	18	38.3	34	1.9	116	19	16.4	22	1.2
White Yam	62	29	46.7	49	1.7	95	44	46.3	83	1.9
Yellow Belmont	67	31	46.3	92	3	90	28	31.1	58	2.1
Yellow Jersey	132	29	21.9	48	1.6	44	22	50	45	2
Yellow Strassburg	56	19	33.9	26	1.4	98	18	18.4	34	1.9

When the number of lesions per potato for the years 1923 and 1924 was determined the potatoes were classed as large, medium, and small. As might be expected, the highest average was found for those of the larger size, being 2.6, 1.9, and 1.5 per potato for the larger, medium, and small sizes, respectively.

If a comparison is made of the percentage of infection of the different varieties, some surprising and striking variations are noted. The percentage of infection varies from 6.8 (Red Jersey, 1924) to 81.5 (Red Brazil, 1923), the average for all varieties being 36.6 for 1923, and 45.9 for 1924. General Grant Vineless, Southern Queen, Big Stem Jersey, and Pierson had a relatively low percentage of infection in 1923. The average percentage of infection in 1924 was higher than in 1923. The fact that such varieties as General Grant Vineless, Southern Queen, and Pierson had a small percentage of infection in 1923 seems to indicate little regarding their resistance, inasmuch as the percentage in 1924 for these same varieties was consistently higher and about equal to the average for all the varieties. It seems that the small amount of black rot on some of the varieties was due to their having escaped infection rather than to resistance. On the other hand it might conceivably be argued that some varieties are a little more susceptible, in view of the high percentage of infection either or both seasons. For example, there were 4.5 lesions per potato and a percentage of infection of 80.5 in the Haiti in 1924. However, in 1923 the average number of lesions per potato, as well as the percentage of infected potatoes, was much lower. Pumpkin had a high percentage of infection both years, and also, on an average, a comparatively large number of spots per potato. Two varieties, Nancy Hall and Porto Rico, deserve special consideration because of their commercial supremacy. In 1924, both these varieties appeared to be very susceptible, but a study of the table shows that several other varieties were equally so, when the results are based on percentages alone or on the number of lesions per potato (Pumpkin, Haiti, Key West, Dahomey), or both. These two varieties, with an average percentage of infection of 75 and 75.9 and 2.6 and 3 lesions per potato, respectively, immediately attracted attention. The lesions were not only large, often attaining a diameter of 3 inches, but were conspicuous in appearance. While the number of lesions per potato of some of the other varieties was quite as large, the spots were smaller and less noticeable. The results seem to indicate that both Porto Rico and Nancy Hall stand high among the popular commercial varieties in degree of susceptibility. On the other hand, there are several varieties not so well known and popular from the commercial standpoint which are quite as susceptible to the disease. The Jersey group, some varieties of which have gained great favor in some States and on certain markets, appear as a whole to be less susceptible than Porto Rico, Nancy Hall, and some of the other varieties which are restricted more or less to the Southern States.

SUMMARY

Twenty-one varieties of sweet potatoes (*Ipomoea batatas* Poir.), including most of the best-known commercial varieties, were tested under field conditions for a period of four years to determine their

resistance or susceptibility to black rot, caused by *Ceratostomella fimbriata* (E. & H.) Elliott.

It was found that although a large percentage of the plants may be diseased when set in the field, the number of black-rot sweet potatoes at harvest time may be relatively small.

Ten days to two weeks after setting the plants in the field a large percentage of the plants was diseased. Frequently the potatoes when dug did not have black rot, so far as the writers were able to determine, even though they were grown on black rot plants.

On the day they were harvested, one bushel of each variety was placed in storage at a temperature and humidity suitable for the development of black rot. Although a low percentage of the potatoes showed black rot when dug, the disease developed abundantly on all varieties in storage. The potatoes for storage were not selected, but were gathered indiscriminately from the field.

These results indicate that none of the varieties included in these experiments are immune to black rot. A variety showing a relatively low percentage of infection one year may be badly diseased the next. The same may also be said with respect to the average number of lesions per potato.

The storage extended over a period of about seven weeks. Some of the most popular varieties (Porto Rico, Nancy Hall) developed large (up to 3 inches) conspicuous lesions.

THE EFFECT OF MOISTURE ON THE LOSS OF SUGAR FROM SUGAR BEETS IN STORAGE[1]

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INTRODUCTION

The effect of temperature on the storage of sugar beets has been studied for years, but the effect of moisture on sugar-beet storage has apparently received little attention. The aim of the work reported in this paper was: (1) To obtain more information bearing on the earlier statements of the writer[2] that sugar beets lose less sugar when stored moist than when stored dry; (2) to determine the specific moisture conditions required by sugar beets during storage in order to decrease to a minimum the loss of sugar from them; and (3) to point out the necessity of special and definite moisture control in the storage of living tissues in general.

METHODS

All these investigations were carried on under constant temperatures. Sugar beets which were healthy and fairly uniform in sugar content were selected for all experiments. The storage periods were long, *i. e.*, about 100 days or more in duration. After storage, all beets which showed signs of decay were discarded before the analysis. The values for sugar are given as sucrose. The order of sampling and weighing the beets was the same as reported earlier by the writer.[3] With this method comparable weights and percentages of sugar were obtained for each beet by analyzing the same individual beets both before and after storage.

INVESTIGATIONS

In 1921, the writer for the first time found that sugar beets lost more sugar under dry than under moist storage. Early in 1922 this was confirmed by a further test in which five beets were packed in air-dry sand, at each of the following constant temperatures: 1.7°, 4.4°, 9.4°, and 14.4° C.; and similar lots of beets were packed in moist sand at the same temperatures. In general, the beets stored in dry sand lost more sugar than the corresponding lots stored in moist sand at each of the different temperatures.

On April 8, 1922, from 2 to 3 kilograms of beets were stored dry at each of the above-stated constant temperatures and similar check lots were stored moist at the same temperatures. Again the average loss was higher for the beets stored dry, though exceptions appeared at both the highest and lowest temperatures. These exceptions, however, were undoubtedly due to variations in moisture content of the sand.

[1] Received for publication, Oct. 15, 1925; issued, June, 1926.

[2] PACK, D. A. THE STORAGE OF SUGAR BEETS. Facts About Sugar 19: 178–180, 208–209, 232–233, 235, 251–253, illus. 1924.

[3] PACK, D. A. TIME FOR TESTING MOTHER BEETS. Jour. Agr. Research 26: 125–150. 1923.

In an experiment started on November 23, 1923, more constant moisture conditions were obtained by packing some beets in cans with 60 per cent saturated moist sand, and others in air dry sand. The cans contained from 8 to 10 beets each. A set of the moist and a set of the dry packed beets were placed at the constant temperatures of 1.7°, 4.4°, 9.4°, and 14.4° C. The average results showing the sugar lost by the moist and by the dry stored beets at each of the constant temperatures are given in Table I.

TABLE I.—*The effect of moisture as a condition of storage on the loss of sugar by sugar beets*

Temperature	Moisture conditions	Sugar content of beets at beginning of storage	Sugar lost	Sugar lost	Temperature	Moisture conditions	Sugar content of beets at beginning of storage	Sugar lost	Sugar lost
° *C.*		*Grams*	*Grams*	*Per cent*	° *C.*		*Grams*	*Grams*	*Per cent*
1.7	Moist	192	22	11.5	9.4	Moist	199	35	17.6
1.7	Dry	190	32	16.9	9.4	Dry	208	75	36.1
4.4	Moist	174	26	14.9	414.4	Moist	186	30	16.1
4.4	Dry	204	51	25.0	414.4	Dry	231	87	37.6

The analyses show consistently that the beets stored in moist sand lost less sugar under each of the constant temperatures than those stored dry.

On November 17, 1922, 64 beets were packed in sand, the moisture content of which was such that its surface tension was in equilibrium with an osmotic pressure of 6.0 atmospheres pressure at 4.4° C., and these beets were designated the moist lot. At this date 80 similar beets were packed in dryer sand, the surface tension of which was in equilibrium with an osmotic pressure of 10.0 atmospheres pressure at 4.4° C., and these beets were designated the dry lot. After 110 days' storage, it was found that the moist lot had lost 12.9 per cent of its sugar content, and the dry lot 16.5 per cent. Thus, over a prolonged period, only a slight increase in the moisture content of the packing medium resulted in a saving of 22 per cent more sugar.

On November 17, 1923, 5 lots of 50 beets each were placed in storage for 103 days under definite moisture conditions at the constant temperature of 4.4° C. Not only were the beets weighed and analyzed individually, both before and after storage, but the surface tension and the per cent of water in the sand were determined for each moisture condition. The detailed figures for these lots, 1, 2, 3, 4, and 5, are given in Tables II, III, IV, V, and VI, respectively. A summary of the results is given in Table VII. Table VIII gives these results reduced to the terms ordinarily used by the beet-sugar industries of Europe and America. These data show definitely that the percentage of sugar lost by beets during storage increases as the dryness of the storage condition increases.

TABLE II.—*Data on beets of lot 1 before and after storage at 4.4° C., and air of 3.76 mm. vapor pressure*

Tag No. of beet	Before storage			After storage				
	Weight of beet	Sugar as sucrose	Sugar content	Weight of beet	Sugar as sucrose	Sugar content	Loss or gain in weight	Sugar lost
	Grams	*Per cent*	*Grams*	*Grams*	*Per cent*	*Grams*	*Grams*	*Grams*
2007	975	18.2	177	700	21.4	150	−275	27
2091	1,280	18.2	233	830	22.6	187	−450	46
2094	1,020	18.2	186	515	27.6	142	−505	44
2102	905	18.4	167	460	30.6	141	−445	26
2119	975	18.4	179	680	23.6	160	−295	19
2120	850	19.6	167	450	29.0	130	−400	37
2135	795	19.2	153	361	26.0	94	−434	59
2162	1,005	18.6	187	650	24.8	161	−355	26
2163	980	18.8	184	507	27.8	141	−473	43
2168	1,110	18.4	204	575	31.0	178	−535	26
2169	1,290	18.0	232	650	31.0	201	−640	31
2200	970	17.6	171	540	22.0	119	−430	52
2209	1,050	17.6	185	730	16.0	117	−320	68
2225	830	19.4	161	520	28.2	147	−310	14
2266	980	18.4	180	680	22.6	154	−300	26
2268	1,340	17.8	238	1,040	19.8	206	−300	32
2277	915	17.8	163	495	19.6	97	−420	66
2284	1,170	18.2	213	750	13.8	103	−420	110
2287	1,415	18.4	260	960	20.6	197	−455	63
2318	1,000	18.2	182	530	29.0	154	−470	28
2329	845	19.0	160	500	28.0	140	−345	20
2354	1,180	18.0	212	840	22.4	188	−340	24
2355	925	17.8	165	430	29.2	125	−495	40
2359	1,435	17.8	255	852	24.6	209	−583	46
2363	1,430	18.2	260	725	31.0	225	−705	35
2367	815	19.4	158	400	31.8	127	−415	31
2371	1,350	17.8	240	575	29.8	171	−775	69
2373	1,090	18.4	200	475	33.4	159	−615	41
2393	915	18.8	172	500	25.6	128	−415	44
2395	810	19.4	157	465	20.2	94	−345	63
2400	1,025	18.0	184	450	30.4	137	−575	47
2403	1,070	18.0	193	490	33.4	164	−580	29
2408	1,550	17.4	270	825	28.0	231	−725	39
2411	1,625	17.0	276	1,000	21.4	214	−625	62
2412	1,680	17.2	289	900	21.2	191	−780	98
2414	1,135	18.0	204	465	34.8	162	−670	42
2415	1,220	18.0	220	890	21.0	187	−330	33
2416	1,230	17.4	214	680	24.2	165	−550	49
2417	835	19.2	160	450	27.6	124	−385	36
2426	1,350	17.4	235	750	25.4	190	−600	45
2432	955	18.0	172	460	33.6	154	−495	18
2434	910	18.4	167	650	23.0	149	−260	18
2439	1,000	18.4	184	670	24.0	161	−330	23
2456	1,015	18.8	191	690	21.8	150	−325	41
2598	725	18.6	135	450	26.8	120	−275	15
2599	1,455	17.6	256	715	29.0	207	−740	49
2606	1,605	17.2	276	870	23.6	205	−735	71
2610	1,125	18.2	205	760	23.6	179	−365	26
2636	895	17.6	158	420	32.0	134	−475	24
2639	1,080	17.6	190	645	25.0	161	−435	29

TABLE III.—*Data on beets of lot 2 before and after storage at 4.4° C., and a moisture condition which was in equilibrium with 132 atmospheres osmotic pressure*

Tag No. of beet	Before storage			After storage				
	Weight of beet	Sugar as sucrose	Sugar content	Weight of beet	Sugar as sucrose	Sugar content	Loss or gain in weight	Sugar lost
	Grams	*Per cent*	*Grams*	*Grams*	*Per cent*	*Grams*	*Grams*	*Grams*
276	1,305	17.6	230	1,250	16.8	208	−55	22
432	1,710	18.0	308	1,650	17.0	281	−60	27
437	1,335	18.4	245	1,285	17.0	219	−50	26
468	1,620	17.6	285	1,460	17.5	256	−160	29
488	1,060	18.6	197	835	17.6	147	−225	50
508	1,640	17.6	289	1,500	14.5	218	−140	71
509	1,125	17.8	200	1,075	17.4	187	−50	13
512	1,190	18.0	214	1,080	13.8	149	−110	65
587	1,170	17.8	208	1,110	17.0	189	−60	19
588	1,770	18.2	322	1,675	15.2	255	−95	67
611	1,470	17.6	259	1,370	16.7	229	−100	30
617	1,360	18.0	245	1,260	16.8	211	−100	34
627	1,470	17.6	259	1,340	16.5	221	−130	38
698	1,370	18.4	252	1,210	18.4	223	−160	29
711	1,730	17.6	304	1,550	17.6	273	−180	31
717	1,380	18.0	248	1,330	18.0	239	−50	9
743	1,330	17.8	237	1,290	16.0	206	−40	31
745	1,540	17.8	274	1,430	15.6	223	−110	51
781	1,220	18.0	220	1,090	18.0	196	−130	24
786	1,390	18.0	250	1,300	17.8	231	−90	19
787	1,625	17.8	289	1,525	16.7	255	−100	34
790	1,160	17.8	206	1,050	17.6	185	−110	21
797	2,130	17.8	380	1,970	18.5	364	−160	16
814	1,100	17.6	193	1,012	16.4	166	−88	27
844	1,415	17.6	249	1,270	13.6	173	−145	76
873	1,400	17.6	247	1,252	19.0	238	−148	9
874	1,240	18.2	226	1,150	16.8	193	−90	33
877	1,830	18.2	333	1,745	17.8	311	−85	22
1069	1,320	18.2	240	1,125	18.9	213	−195	27
1159	1,700	17.6	299	1,620	15.8	256	−80	43
1183	1,350	17.6	237	1,280	17.2	220	−70	17
1185	1,160	17.6	204	1,090	18.0	196	−70	8
1190	1,380	17.8	246	1,240	17.6	218	−140	28
1210	1,450	18.0	261	1,375	15.2	209	−75	52
1275	1,390	18.2	253	1,345	16.4	221	−45	32
1287	1,620	18.0	292	1,550	18.0	279	−70	13
1292	1,730	17.6	305	1,625	17.6	286	−105	19
1300	1,520	18.0	274	1,360	15.8	215	−160	59
1358	1,130	18.0	203	1,050	15.7	165	−80	38
1316	1,100	18.0	198	1,050	17.2	181	−50	17
1340	1,320	18.0	237	1,100	12.2	134	−220	103
1365	1,125	18.4	207	1,025	17.9	183	−100	24
1429	1,875	17.0	319	1,715	14.6	250	−160	69
1433	1,360	17.4	237	1,240	17.2	214	−120	23
1437	1,550	17.8	276	1,450	17.6	255	−100	21
1439	1,240	17.6	218	1,150	18.0	207	−90	11
1443	1,425	18.0	257	1,380	17.4	240	−45	17
1444	1,360	17.8	242	1,275	18.1	230	−85	12
1446	1,350	17.8	241	1,275	18.0	229	−75	12
1451	1,620	17.6	285	1,455	17.4	253	−165	32

TABLE IV.—*Data on beets of lot 3 before and after storage at 4.4° C., and a moisture condition in equilibrium with 35.7 atmospheres osmotic pressure*

Tag No. of beet	Before storage			After storage				
	Weight of beet	Sugar as sucrose	Sugar content	Weight of beet	Sugar as sucrose	Sugar content	Loss or gain in weight	Sugar lost
	Grams	*Per cent*	*Grams*	*Grams*	*Per cent*	*Grams*	*Grams*	*Grams*
47	1,250	17.6	220	1,185	16.8	199	−65	21
242	1,630	17.8	290	1,550	16.2	251	−80	39
325	1,440	18.4	265	1,420	16.8	239	−20	26
374	1,155	17.6	203	1,125	16.9	190	−30	13
445	1,270	18.2	231	1,220	17.6	215	−50	16
449	1,370	18.0	247	1,310	16.6	217	−60	30
450	1,250	18.2	227	1,220	17.4	212	−30	15
442	1,680	17.6	295	1,580	16.0	253	−100	42
472	1,920	17.8	342	1,780	16.6	295	−140	47
476	1,725	18.4	317	1,640	16.4	269	−85	48
517	1,340	18.2	244	1,320	16.0	211	−20	33
555	1,580	18.4	293	1,550	17.0	264	−30	29
535	1,540	17.8	274	1,400	16.6	232	−140	42
566	1,075	18.4	198	1,050	17.5	184	−25	14
578	1,490	18.0	268	1,450	16.6	241	−40	27
582	1,500	18.0	270	1,475	17.6	261	−25	9
598	1,680	18.0	302	1,600	17.4	278	−80	24
626	1,220	17.8	217	1,100	15.4	169	−120	48
639	1,275	17.8	227	1,215	16.4	199	−60	28
647	1,690	17.6	297	1,600	16.8	269	−90	28
653	1,770	18.2	322	1,700	17.4	295	−70	27
685	1,375	18.0	247	1,230	15.6	192	−145	55
691	1,080	18.0	194	1,030	18.0	185	−50	9
727	1,220	18.4	225	1,140	17.2	196	−80	29
864	1,830	18.2	333	1,825	16.2	296	−5	37
878	1,430	18.0	257	1,366	16.3	222	−64	35
1052	1,430	17.8	254	1,380	17.0	235	−50	19
1108	1,060	18.2	193	1,050	18.0	189	−10	4
1146	1,550	17.8	276	1,525	16.0	244	−25	32
1154	1,350	18.4	249	1,280	12.8	164	−70	85
1207	1,230	17.6	217	1,090	16.2	177	−140	40
1211	1,200	17.6	211	1,080	15.4	166	−120	45
1216	1,160	17.8	206	1,160	16.2	188	0	18
1226	1,250	18.4	230	1,240	16.4	203	−10	27
1228	1,290	17.8	229	1,250	16.6	208	−40	21
1223	1,420	17.8	253	1,454	17.0	247	+34	6
1231	1,200	18.4	221	1,250	15.2	190	+50	31
1239	1,900	17.6	335	1,800	17.6	317	−100	18
1245	1,500	18.0	270	1,555	16.0	249	+55	21
1253	1,260	17.8	224	1,170	12.6	147	−90	77
1257	1,070	18.4	197	1,145	17.2	197	+75	0
1270	1,220	18.2	222	1,300	14.6	190	+80	32
1271	1,350	18.0	243	1,330	16.4	218	−20	25
1276	1,590	18.0	286	1,550	17.0	266	−40	22
1293	1,375	18.2	250	1,340	16.6	222	−35	28
1308	1,500	18.2	273	1,465	17.0	249	−35	24
1311	1,670	17.4	291	1,775	14.8	262	+105	29
1312	1,210	18.2	220	1,175	17.2	202	−35	18
1313	1,180	18.4	217	1,250	15.4	193	+70	25
1326	1,225	17.8	218	1,235	16.7	206	+10	12

TABLE V.—*Data on beets of lot 4 before and after storage at 4.4° C., and a moisture condition in equilibrium with 7.6 atmospheres osmotic pressure*

Tag No. of beet	Before storage			After storage				
	Weight of beet	Sugar as sucrose	Sugar content	Weight of beet	Sugar as sucrose	Sugar content	Loss or gain in weight	Sugar lost
	Grams	*Per cent*	*Grams*	*Grams*	*Per cent*	*Grams*	*Grams*	*Grams*
230	1,275	18.2	232	1,280	17.0	218	+5	14
260	1,170	18.2	212	1,210	15.2	184	+40	28
355	1,215	18.4	223	1,175	17.6	208	−40	15
448	1,160	18.8	218	1,180	16.6	193	0	25
478	1,970	18.2	359	2,050	15.0	308	+80	51
484	1,480	17.6	260	1,555	16.4	255	+75	5
496	1,250	17.8	223	1,320	15.6	206	+70	17
504	1,490	17.6	262	1,520	16.6	252	+30	10
510	1,200	17.8	214	1,300	15.4	200	+100	14
554	1,575	17.6	277	1,440	16.0	230	−135	47
563	1,540	18.4	283	1,500	17.6	264	−40	19
564	1,330	17.6	234	1,315	15.5	204	−15	30
575	1,430	17.8	254	1,500	14.0	210	+70	44
616	1,375	18.0	247	1,460	15.2	222	+85	25
619	1,160	18.4	213	1,230	15.6	192	+70	21
620	1,340	18.0	241	1,375	16.4	225	+35	16
641	1,410	18.0	253	1,450	16.1	233	+40	20
651	1,870	18.0	337	1,900	16.6	315	+30	22
657	1,125	18.2	205	1,150	15.4	177	+25	28
678	1,225	18.0	220	1,220	16.0	195	−5	25
700	1,860	18.2	339	1,750	16.2	284	−110	55
701	1,570	17.6	276	1,550	15.8	245	−20	31
784	1,175	18.4	216	1,160	18.0	209	−15	7
839	1,440	17.8	256	1,475	15.8	233	+35	23
859	1,320	17.6	232	1,325	16.0	212	+5	20
879	1,330	17.8	237	1,375	16.8	231	+45	6
899	1,250	17.6	220	1,230	16.6	204	−20	16
947	1,300	17.6	229	1,315	15.5	204	+15	25
985	1,700	17.8	303	1,735	16.5	286	+35	17
986	1,430	17.6	252	1,360	17.2	234	−70	18
1014	1,520	17.6	267	1,550	15.9	247	+30	20
1017	1,620	18.0	292	1,650	16.3	269	+30	23
1033	1,380	18.2	251	1,400	16.9	237	+20	14
1044	1,075	17.6	189	1,120	15.2	170	+45	19
1048	1,770	17.6	311	1,830	16.0	293	+60	18
1058	1,370	18.2	249	1,350	16.8	227	−20	22
1061	1,390	18.4	256	1,470	16.2	238	+80	18
1063	1,960	17.8	349	1,990	16.0	318	+30	31
1065	1,600	17.6	282	1,660	15.2	252	+60	30
1092	1,580	18.0	285	1,630	16.0	261	+50	24
1107	1,430	18.0	257	1,440	16.6	239	+10	18
1110	1,790	17.8	319	1,825	16.6	303	+35	16
1112	1,400	17.8	249	1,420	15.6	222	+20	27
1122	1,380	17.6	243	1,425	15.6	222	+45	21
1128	1,530	18.2	279	1,630	16.0	261	+100	18
1166	1,150	19.2	221	1,210	16.0	194	+60	27
1277	1,250	18.2	227	1,340	16.5	221	+90	6
1302	1,500	18.4	273	1,550	17.4	270	+50	3

TABLE VI.—*Data on beets of lot 5 before and after storage at 4.4° C., and a moisture condition in equilibrium with 4.4 atmospheres osmotic pressure*

Tag No. of beet	Before storage			After storage				
	Weight of beet	Sugar as sucrose	Sugar content	Weight of beet	Sugar as sucrose	Sugar content	Loss or gain in weight	Sugar lost
	Grams	*Per cent*	*Grams*	*Grams*	*Per cent*	*Grams*	*Grams*	*Grams*
2008	870	19.2	167	906	16.0	145	+36	22
2010	1,140	17.6	201	1,216	14.4	175	+76	26
2021	840	19.0	159	941	15.6	147	+101	12
1026	800	18.8	150	891	15.6	139	+91	11
2058	1,040	18.0	187	1,206	13.4	162	+166	25
2074	840	19.4	163	926	16.0	148	+86	15
2088	925	18.6	172	1,026	15.4	158	+101	14
2098	1,060	18.0	191	1,131	15.4	174	+71	17
2136	865	19.2	166	956	14.6	140	+91	26
2139	840	19.4	163	941	16.8	158	+101	5
2166	1,335	17.8	238	1,441	14.2	205	+106	33
2178	975	17.6	172	1,111	14.2	158	+136	14
2181	890	19.0	169	951	16.6	158	+61	11
2188	1,110	17.8	198	1,176	15.8	186	+66	12
2193	1,050	18.2	191	1,091	15.4	168	+41	23
2211	780	19.2	150	856	17.0	146	+76	4
2220	930	18.8	175	1,041	15.0	156	+111	19
2248	1,160	17.8	206	1,201	15.0	180	+41	26
2267	860	19.2	165	921	15.2	140	+61	25
2295	870	19.4	169	956	15.8	151	+86	18
2321	895	18.6	166	951	14.6	139	+56	27
2341	1,070	18.0	193	1,146	14.4	165	+76	28
2346	1,080	18.0	194	1,201	15.2	183	+121	11
2350	1,025	18.0	185	1,096	15.2	167	+71	18
2351	860	17.6	151	921	15.0	138	+61	13
2398	915	18.0	165	1,136	13.0	148	+221	17
2401	1,050	18.2	191	1,126	15.2	171	+76	20
2404	560	19.0	106	626	15.4	96	+66	10
2410	1,375	17.6	242	1,416	15.8	224	+41	18
2435	1,060	18.0	191	1,166	16.0	187	+106	4
2436	1,165	17.6	205	1,261	13.8	174	+96	31
2437	800	18.8	150	861	16.4	141	+61	9
2449	830	19.4	161	951	15.6	148	+121	13
2512	1,000	17.8	178	1,116	13.2	147	+116	31
2514	1,080	18.4	199	1,241	15.2	189	+161	10
2515	1,100	18.4	202	1,166	14.8	173	+66	29
2519	1,050	17.4	183	1,261	14.2	179	+211	4
2522	860	18.8	162	946	13.8	131	+86	31
2523	1,560	17.2	268	1,731	14.8	256	+171	12
2525	785	18.6	146	841	12.2	103	+56	43
2529	1,160	18.0	209	1,351	14.0	189	+191	20
2533	1,290	17.0	219	1,501	13.8	207	+211	12
2534	910	18.4	167	1,046	14.4	151	+136	16
2535	1,195	18.2	218	1,261	15.0	189	+66	29
2536	1,165	18.0	209	1,251	14.8	185	+86	24
2544	950	18.2	173	1,101	15.2	167	+151	6
2594	900	17.8	160	951	15.8	150	+51	10
2613	1,400	17.4	244	1,426	14.6	208	+26	36
2617	960	17.6	169	976	15.6	152	+16	17
2632	1,015	17.4	177	1,112	13.6	151	+97	26

TABLE VII.—*Per cent loss of sugar accompanying loss or gain in weight of sugar beets during storage, as effected by various moisture conditions of storage*

Condition of storage	Osmotic pressure [a]	Water in storage air and sand	Before storage		After storage		Average loss or gain in weight	Average loss or gain in weight	Average sugar lost	Average loss of sugar
			Average weight of beet	Average sugar content	Average weight of beet	Average sugar content				
	Atmosphere	*Per cent*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Per cent*	*Grams*	*Per cent*
Very dry	(b)	[c] 0.36	1,103	200	632	159	−471	−42.7	41	20.5
Dry	132.0	1.20	1,423	254	1,317	222	−106	−7.4	32	12.6
Medium	35.7	1.64	1,400	252	1,359	223	−41	−2.9	29	11.5
Moist	7.6	4.87	1,430	257	1,456	235	+26	+1.8	22	8.6
Very moist	4.4	8.87	1,005	183	1,100	164	+95	+9.5	19	10.4

[a] Osmotic pressure in atmospheres which was found to be at equilibrium with the surface tension of the water in the sand.

[b] This value would probably correspond to several hundred atmospheres.

[c] Per cent of moisture in air.

TABLE VIII.—*Relative amounts of sugar lost by beets accompanying the loss or gain in weight of sugar beets during 103 days' storage at 4.4° C. and under various moisture conditions*

[The results are calculated from Tables II, III, IV, V, VI, and VII]

Before storage		After storage					
Weight of beets	Weight of sugar in beets	Condition of storage	Loss or gain in weight of beets during storage	Sugar lost during 103 days' storage	Sugar lost per 100 kg. of beets	Sugar lost per 100 kg. of beets per day	Sugar lost per ton of beets per day
Kilogram	*Kilogram*		*Per cent*	*Kilogram*	*Kilogram*	*Kilogram*	*Pounds*
55.135	9.980	Very dry	−42.7	2.050	3.7181	0.0361	0.722
71.160	12.700	Dry	−7.4	1.600	2.2484	.0218	.436
69.975	12.590	Medium	−2.9	1.430	2.0436	.0198	.396
68.660	12.326	Moist	+1.8	1.049	1.5277	.0148	.296
50.245	9.135	Very moist	+9.5	.933	1.8569	.0180	.360

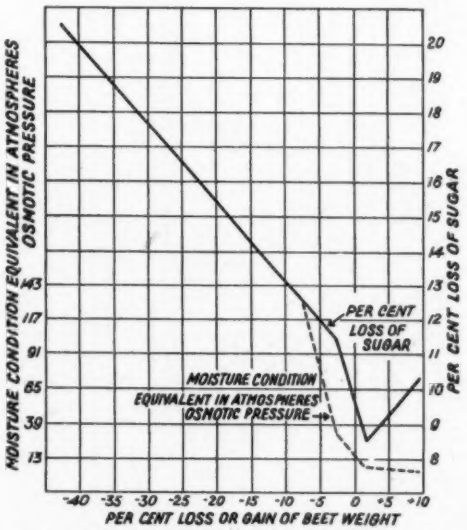
MOISTURE CONDITION EQUIVALENT IN ATMOSPHERES OSMOTIC PRESSURE
PER CENT LOSS OF SUGAR
PER CENT LOSS
OF SUGAR
MOISTURE CONDITION
EQUIVALENT IN ATMOSPHERES
OSMOTIC PRESSURE
PER CENT LOSS OR GAIN OF BEET WEIGHT
-40 -35 -30 -25 -20 -15 -10 -5 0 +5 +10
20 19 18 17 16 15 14 13 12 11 10 9 8
143 117 91 65 39 13

FIG. 1.—Graphs showing the relation between the per cent gain or loss in weight of sugar beets, and the per cent loss of sugar from them, during storage

Figure 1 shows the relation between the per cent gain or loss of weight of beets and the per cent loss of sugar during storage. These values (taken from Table VII) indicate that the smallest loss occurs when the beets retain their normal amount of water. The normal water content was maintained by keeping the moisture in the packing material equivalent to, or a little higher than that of normal beet tissues.

The values for the mean and standard deviation of per cent loss of sugar, the per cent loss or gain in weight of the beets in lots 1, 2, 3, 4, and 5, and the differences for lots 1 and 4, are given in Table IX. These data were calculated from the per cent loss or gain for each

beet. The results show, as was earlier reported by the writer[4] that individual beets are irregular as to the per cent loss of sugar and the per cent change in weight during storage. These irregularities are due to individual characteristics of the beets and their condition at the time of storing. Some of the causes of these irregularities in weight were earlier reported by the writer.[5] The differences between the amount of sugar lost and the changes in weight of the beets of lots 1 and 4 are significant and of value to the industry.

TABLE IX.—*The mean and standard deviation values for the per cent loss of sugar and the per cent loss or gain in weight of the five lots of beets given in Tables II, III, IV, V, and VI*

Lot No.	Per cent loss of sugar by beets		Per cent loss or gain in weight of beets	
	M	σ	M	σ
1	20.48±0.87	9.16±0.62	−42.94±0.87	9.14±0.62
2	12.82± .75	7.83± .53	−7.58± .34	3.54± .24
3	11.28± .62	6.54± .44	−2.82± .35	3.67± .25
4	8.56± .35	3.63± .25	+1.83± .33	3.40± .23
5	10.58± .60	6.29± .42	+9.68± .43	4.54± .30
Difference, 1–4	11.92± .94		44.77± .93	

To determine the limit of the beneficial effect of increased moisture during storage, beets were submerged in water at 4.4° C. for 164 days. These beets lost an average of 55 per cent of their sugar. Some fermentation had started before the end of this period. The excessive loss of sugar in this case apparently was due to lack of aeration rather than to the increased supply of water. This was substantiated in further tests in which the sugar beets were abundantly supplied with free water, but were well aerated. Here the loss in sugar was appreciably less.

To determine the applicability of these results to commercial practice, several hundred pounds of beets were stored 43 days, during the fall of 1924, in bins corresponding to sections of commercial sugar-beet piles. The beets of one bin were stored just as in the ordinary storage pile. Those of a second bin were similarly stored, except that they were kept moist. As reported in an earlier publication,[6] the beets as ordinarily stored lost 75 per cent more sugar than did the beets which were kept moist.

DISCUSSION

In the commercial storage of beets, the sugar losses and chemical changes resulting from the physiological processes of the living and healthy stored beets receive attention. It should be clearly understood that the storage of rotten, diseased, or dead beets is not under consideration here, and furthermore the ordinary methods of food preservation by means of heat, chemicals, sugar in high concentrations, drying, or freezing are not effective. In this connection it should be remembered that when sugar beets are killed by chemicals, heat, or freezing the loss of sugar and decomposition processes

[4] PACK, D. A. TIME FOR TESTING MOTHER BEETS. Jour. Agr. Research 26: 125–150. 1923.
[5] PACK, D. A. THE STORAGE OF MOTHER BEETS. Facts About Sugar 20: 874–875. 1925.
[6] PACK, D. A. STORAGE OF COMMERCIAL BEETS. Through the Leaves 13: 157–159, 206–207, 272–274, illus. 1925.

proceed rapidly, unless careful protection is afforded. To carry these treatments to a point where they would stop all biological and catalytic action on so large an amount of material, even if otherwise practicable, would be too costly. Under such difficulties, sugar beets must be kept alive in storage.

The results of the present investigation show that in order to conserve the maximum amount of sugar, the conditions of storage must be so controlled that the water content of the beet is kept normal. On the other hand, the beet seems to be under optimum conditions for growth and respiration, when it contains its normal amount of water. These processes, of course, tend to consume the reserve sugar of the beet, but the difficulty is overcome by subjecting the beet of normal water content to a low temperature. The writer[7] has found that 1.7° C. would inhibit growth and reduce to a minimum the respiration of normal sugar beets; thus the life processes which use up the reserve sugar are inhibited or slowed up. The writer has kept sugar beets healthy and at a high sugar content for 18 months under these conditions. Even though reserve food is slowly used up by these retarded physiological processes, the loss is near the minimum, and in the development of the dormant habit in nature an economical storage method, at least for living tissues, has been evolved. It is interesting to note that in order to reduce to a minimum the sugar lost by beets during storage, the tissues must not only be kept alive, but they must be kept as dormant as possible.

SUMMARY

These investigations indicate the importance of moisture in the storage of sugar beets, and show that moist-stored sugar beets lose less sugar than similar sugar beets stored dry.

The loss of sugar by sugar beets increases as the degree of dryness of the storage conditions increases; thus dry storage accelerates while moist storage retards this loss of sugar.

The specific moisture conditions required by sugar beets during storage, in order to reduce to a minimum their loss of sugar, is a moisture condition which is in equilibrium with the water content of the beet tissues. This moisture condition is equivalent to about 12 atmospheres osmotic pressure.

The sugar losses of commercial sugar beets, under practical conditions of storage, were decreased from 40 to 50 per cent by moist storage.

Sugar beets should be kept alive in storage. The best results are obtained when the tissues of these living beets have normal water content, are healthy, properly aerated, and dormant. Dormancy under such conditions may be brought about by low temperatures.

[7] PACK, D. A. THE STORAGE OF SUGAR BEETS. Facts About Sugar 19: 178–180, 208–209, 232–233, 235, 251–253, illus. 1924.

INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE ON THE INFECTION OF SWEET POTATOES BY THE BLACK-ROT FUNGUS[1]

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INTRODUCTION

The occurrence of epidemics of plant diseases during certain seasons has been quite generally recognized. That these epidemics are to some extent dependent upon certain weather conditions seems certain, and data have been offered in particular instances to explain them. When the disease is of the leaves or stems the epidemic is accounted for in many cases by certain environmental conditions, such as the temperature and humidity of the air. If the disease is of the underground parts of the plant, unfavorable soil temperature and moisture may be offered as an explanation. Besides the temperature and moisture of the soil, the physical properties of the soil seem to be closely associated with certain diseases; for example, some of the wilt diseases, and particularly those caused by Fusaria, seem to be worse on sandy soils. Monteith [2] found that soil temperature was not a limiting factor in the infection of cabbage by *Plasmodiophora brassicae* Wor., infection occurring over a wide range of temperatures. On the other hand, he found that clubroot developed readily in a soil with a moisture content of 60 per cent and more of its water-holding capacity. No clubroot occurred at 45 per cent of the water-holding capacity of the soil, and yet a soil moisture of 45 per cent was sufficient to grow a good crop of cabbage. Poole [3] found that sweet potatoes (*Ipomoea batatas* Poir.) were severely attacked by the soil-rot (pox) organism in very dry soils, but not at all in wet soils. Other investigators have demonstrated a similar relationship of the soil moisture and temperature to infection of certain crops by plant pathogens.

The black rot of sweet potatoes caused by *Ceratostomella fimbriata* (E. & H.) Ell. is a common and destructive disease, often occurring abundantly in the hotbed if diseased potatoes are used for seed or if the seed potatoes are bedded in infested soil. Observations have indicated that when only a very limited amount of water was applied to the soil the disease was not so prevalent or severe. If these observations were correct, a reduction in the amount of the disease could be expected if water beyond that actually needed to grow the plants was withheld.

With this idea in mind the writers undertook a series of experiments designed to determine the relation of the temperature and moisture of the soil to infection of sweet potatoes by the black-rot fungus.

[1] Received for publication Nov. 11, 1925; issued June, 1926.

[2] MONTEITH, J., JR. RELATION OF SOIL TEMPERATURE AND SOIL MOISTURE TO INFECTION BY PLASMODIOPHORA BRASSICAE. Jour. Agr. Research 28: 549–562, illus. 1924.

[3] POOLE, R. F. THE RELATION OF SOIL MOISTURE TO THE POX OR GROUND ROT DISEASE OF SWEET POTATOES. Phytopathology 15: 287–293, illus. 1925.

The experiments were undertaken with the hope that a temperature and a moisture content of the soil could be found which would give a minimum of infected plants in the hotbed and thereby reduce the amount of loss in the field.

GENERAL METHODS

All these experiments were carried out in the greenhouse by the use of the so-called Wisconsin soil-temperature tanks, which have been full described by Jones.[4] Each of these tanks was equipped with a propeller to keep the water in constant motion. It was found that during summer weather, in particular, a stratum of warm water would collect on the surface, thereby causing considerable fluctuation in the temperature of the water. Each tank was provided with an independent automatic electric control and could be adjusted to any temperature desired.

The soil used was a light sandy loam obtained from the Arlington Experiment Farm near Rosslyn, Va., and was well suited for sweet potatoes. Enough soil was obtained for the entire series of experiments. Before using it was thoroughly mixed and then run through a sieve to remove pebbles and undecayed plant parts. The soil was rather low in organic matter. The same soil was used several times throughout the experiments. At the termination of an experiment the soil and cans were sterilized by steam for 7 hours at 15 pounds pressure. The soil was again thoroughly mixed and run through a sieve.

The Yellow Jersey variety of sweet potato was used in all the experiments. The potatoes, carefully selected and free of black rot, were bedded in sand. After the plants had attained a height of from 6 to 8 inches they were pulled and the roots dipped in a spore suspension of the black-rot organism which was isolated in 1921 from material from the Azores Islands and kept in a vigorous state of growth by frequent transfers to cooked cornmeal in flasks. The plants were then set in the soil, and water in the required amount was added. Only strong, healthy, well-rooted plants were used. The control plants were in every way treated the same as the inoculated ones, except that they were dipped in tap water instead of in a spore suspension.

SOIL TEMPERATURE IN RELATION TO INFECTION BY THE BLACK-ROT FUNGUS

An equal amount (3,650 grams) of soil, the water-holding capacity of which was determined at the beginning of each experiment, was placed in each can, which filled it to about 1.5 inches from the top. The soil moisture was adjusted by the addition of water, based on the dry weight of the soil. A one-half inch glass tube was inserted about 5 inches into the soil of each of the cans, for the delivery of water about the roots of the plants. Eight cans were used, each containing two to three plants. Two of the cans were held as controls. In no case did any control plants become infected. After the plants were set the required amount of water was added, and then 35 grams of finely ground cork was spread over the surface to reduce evaporation.

[4] JONES, L. R. EXPERIMENTAL WORK ON THE RELATION OF SOIL TEMPERATURE TO DISEASE IN PLANTS. Wis. Acad. Sci., Arts, and Letters, Trans. 20: 433–459, illus. 1921.

The different experiments varied somewhat in duration. At the outset the time required for infection to take place was not known, so some of the preliminary experiments were continued longer than was actually necessary. Where the temperature and moisture of the soil were favorable, conspicuous lesions were produced in five to six days.

The soil employed in these experiments had a water-holding capacity of about 25 per cent (average of five samples), a moisture equivalent of 9 per cent, and a wilting coefficient of 4.9 per cent.

The relation of soil temperature to infection of sweet-potato plants by *Ceratostomella fimbriata* was determined by the use of six tanks, the temperatures of which formed a series from 10° to 35° C. in steps of 5°. These temperatures were employed in all the experiments, which were repeated several times. One tank was run alone at a temperature of 32.5° C. for the purpose of fixing more definitely the upper limit for infection. The temperature of the tanks did not always remain constant, but fluctuated slightly above or below that to which any particular tank was adjusted. If the temperature was found to be too high or too low, it was promptly adjusted to normal by the addition of cold or warm water.

Evidence of infection was apparent in a few days by the sickly appearance of the plants. The plants remained small and showed evidence of stunting and yellowing of the leaves. When the disease was severe the plants wilted and finally died. The most characteristic single symptom of black rot was a yellowing of the foliage, which is characteristic of the disease, as it has been observed in hotbeds and in the field.

About five days after the experiments were started one or two plants in the different tanks were pulled up in order to study the progress of the disease. The lesions on the plants were usually conspicuous in five days, and if the temperature was favorable for the development of the organism the entire root system might be dead in seven or eight days. Figure 1 shows the temperatures employed and the percentage of infected plants at the different temperatures. It should be emphasized at this point that the figure shows merely the percentage of plants infected at a given temperature, and does not take into account the severity of infection, which will be discussed later. Figure 1 shows infection as follows: 10° C., 17 per cent; 15° to 30°, 100 per cent; 32°, 89 per cent; and 35°, none. There is shown a wide range of temperatures over which infection will take place. So far as this disease is concerned the minimum temperature is of little consequence, inasmuch as the organism will infect at a temperature (10° C.) too low for the growth of the plant. As a matter of fact, judging from the results of these experiments, sweet-potato plants will actually die if held at this temperature for any considerable length of time. On the other hand, no infection occurred at 35°, and yet the plants thrived at this temperature. This temperature, if maintained constantly in the hotbed, would reduce if not eliminate black rot. The minimum temperature for infection is below 10°; the optimum, taking the percentage of plants infected as a criterion, covers a wide range of temperature of from about 15° to 30°; the maximum is somewhere between 32.5° and 35°.

A study of the data with respect to the amount of disease shows that the optimum would cover a much narrower range of temperatures. At the close of each experiment all the remaining plants from each tank were pulled up and examined, in order to ascertain the relative amount of disease at the different temperatures. In some cases some of the plants were examined before the termination of the experiment. The results showed that the amount of disease increased with the rise in temperature up to about 25° C. Beyond that point the amount of infection decreased with the rise in temperature, and became nil at 35°. At the optimum (which, from the standpoint of the amount of disease, is about 25°) the entire root system of many plants was affected, the roots being almost completely decayed to the surface of the soil. At lower temperatures in particular, and to a less degree at the higher ones, the number of infection centers were fewer

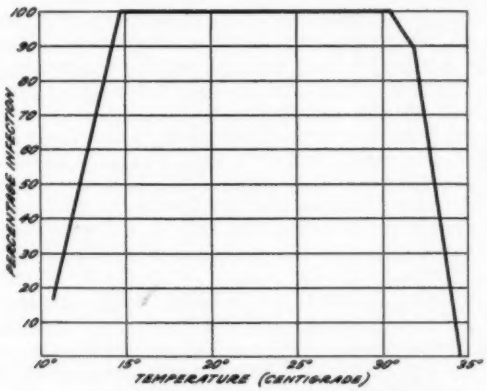

FIG. 1.—Percentage of infection of sweet-potato plants at different temperatures by the black-rot fungus, *Ceratostomella fimbriata*

and smaller than at the optimum. At high temperatures, as for example 32.5°, the spots were small, grayish, slightly sunken, and somewhat water-soaked in appearance, which was quite in contrast to the dark, almost black, lesions which are found on the roots under optimum conditions for infection.

SOIL MOISTURE IN RELATION TO INFECTION BY THE BLACK-ROT FUNGUS

The experiments on the relation of soil moisture to infection were carried out in about the same manner as those with temperature. Four tanks were employed, each containing a different percentage of soil moisture. After the soil was sterilized it was air-dried. The water-holding capacity of the soil and the amount of water still remaining in it were determined. A definite amount of soil was weighed out for the eight pots of each tank and water was added to bring the soil to the desired moisture content. The water-holding capacity of the soil was determined at the beginning of the experiment, and the percentage of the water-holding capacity of the soil of each tank was obtained at the beginning and close of the experiment.

No water was added to the cans during the experiment; the water content of the soil at the end of the experiment was therefore less than at the beginning. After some preliminary experiments and observations it was believed that more reliable data could be obtained by this method than by adding water periodically to the soil. While the addition of water to the soil might maintain the water proportionate to the soil as a whole, the writers have not yet learned of any method whereby it can be uniformly distributed throughout the soil. All the methods now known merely deliver the water to definite localized areas, in which case the percentage of moisture in some parts of the can would necessarily be greater than in others. In other words, some of the roots of the plants might be embedded in saturated soil and others in soil that is very dry.

In view of the fact that infection takes place in a relatively short time and results can be obtained in a few days, the supplying of water beyond that actually contained in the soil at the outset is not so essential. At least it is believed that the results will give a more accurate picture of the relation of moisture to infection than if water was added periodically. The soil was finely pulverized and thoroughly mixed. In order to reduce evaporation to a minimum, 50 grams of ground cork was spread over the surface. All the tanks were adjusted to 25° C., as this temperature had been shown to be the optimum for infection. There were slight fluctuations above and below this temperature.

Each tank contained eight cans, two of which were used as controls, the plants of which in no case became infected. Three plants were set in each can.

The soil-moisture experiments were run three times. It was not possible in any case to obtain exactly the same percentage of moisture in the soil for the different experiments. While a definite amount of soil was employed for each experiment and the theoretical amount of water required was added, the soil (air dried) differed at the beginning in moisture content. The percentage of the water-holding capacity of the soils at the beginning and close of the experiments in the different tanks is listed in Table I, without regard to any particular experiment. The percentage of infected plants is also shown.

TABLE I.—*Percentage of soil moisture and water-holding capacity of the soil at the start and close of the experiment, together with percentage of infection*

Per cent of water in soil		Per cent of water-holding capacity		Per cent of infection
Start	End	Start	End	
2.9	2.3	14.00	10.95	0.0
4.3	3.7	18.20	15.95	100.0
5.9	4.9	25.10	20.95	100.0
5.2	4.0	27.00	20.90	100.0
7.7	6.3	33.00	27.20	100.0
7.9	5.1	40.99	26.60	100.0
10.0	9.4	43.10	40.40	100.0
9.6	8.0	46.00	38.40	100.0
9.0	6.4	47.00	33.40	100.0
11.1	8.4	57.80	43.30	100.0
14.5	10.9	61.96	46.80	100.0
13.4	10.2	69.70	52.90	100.0
16.4	12.4	78.10	59.10	100.0
20.9	17.1	100.00	81.90	100.0

Table I and Figure 2 show that 100 per cent infection took place over an initial range of soil moisture from 18.2 per cent of the water-holding capacity to saturation. It should be emphasized that these results have to do merely with the percentage of infected plants and not with the amount of disease. At 14 per cent of the water-holding capacity of the soil none of the plants became diseased. At the lowest soil-moisture content, when no infection occurred, the plants died from the lack of water. The results show that wherever there is enough moisture to support plant growth infection will occur. At a water-holding capacity of 18.2 per cent the control plants wilted considerably in the sun and made no growth. In general it may be stated that the amount of infection increases with the increase in the percentage of soil mositure up to about 57 to 60 per cent of its water-holding capacity. The amount of infection in the saturated soil was less than at approximately 60 per cent of the water-holding capacity, which as a result of these experiments must be regarded as about the optimum.

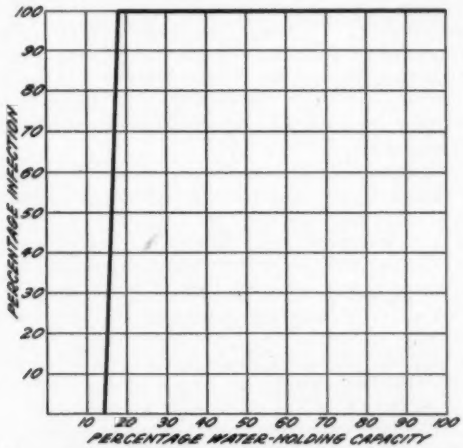

FIG. 2.—Percentage of infection of sweet-potato plants in different percentages of the water-holding capacity of the soil by the black-rot fungus, *Ceratostomella fimbriata*

INFECTION OF SPROUTS FOLLOWING GERMINATION

The experiments thus far have dealt with the relation of temperature and the moisture content of the soil to infection of the plants, the roots of which were dipped in a spore suspension of the black-rot organism. The plants were several weeks old and the roots were more or less wounded and mutilated in the process of pulling from the mother potato, dipping, and planting. Plants so handled became infected over a considerable range of temperature and soil moisture. It was thereby found that wounded plants will become infected if exposed to certain environmental conditions. The danger of using infested soil in the hotbed has been recognized, and the use of clean seed and noninfested soil has been recommended. Although it has been generally accepted that infection of the unwounded sweet-potato sprouts may occur in infested soil, adequate proof has been

lacking. A series of experiments were therefore undertaken in which unwounded sprouts were subjected to infection. These experiments were conducted in the so-called Wisconsin constant-temperature tanks at a fixed temperature and known soil moisture. The potatoes were carefully selected for freedom from black rot, and were handled with extreme care so as not to wound them. They were dipped in a spore suspension of the black-rot organism and then bedded in a soil containing about 60 per cent of its water-holding capacity. The water content of the soil was maintained by bringing the cans to a known weight by the addition of water every one or two days. Water was added more frequently after the vines grew large in order to replace that lost by transpiration. In the first set of experiments the temperature of the soil was maintained at 25° C. This temperature was a little low, which, coupled with the high water content of soil, resulted in the rotting of some of the potatoes before germination took place. In subsequent experiments the temperature was raised to 27.5° C. and the moisture of the soil was reduced. Under these conditions the plants germinated within a few days and produced a good crop of sprouts. The soil was sterilized by steam for 7 hours at 15 pounds pressure before using, and it was assumed that all organisms were killed. During the experiment extreme care was taken not to wound any of the plants. As a matter of fact they were not handled. The cans were lifted from the tanks and weighed at frequent intervals, and water was added, but the plants were not molested.

The experiments were continued for from four to five weeks. The potatoes with the plants attached were then lifted from the cans, the dirt was carefully removed, and the roots were examined for black-rot lesions. The number of sprouts varied from 9 to 21 to the potato. After totaling the number of healthy and diseased plants, it was found that 48.2 per cent of the plants were marked with the characteristic black-rot lesions.

The method of conducting these experiments did not strictly duplicate natural hotbed conditions, in which the fungus is distributed in the soil. Inasmuch, however, as the purpose of the experiments was to prove infection without wounding, it makes little difference whether the organism was previously an inhabitant of the soil or whether it was applied by dipping the potatoes in a spore suspension.

Contrary to what might be expected, infection frequently occurred at some place quite remote from the place of attachment of the sprout to the mother potato. Soil infection was quite general. Numerous infections occurred on the potatoes. Some of the spots were typical of storage-house infections, and in the course of five weeks they attained a diameter of from 0.5 to 1.5 inches.

RELATION OF TEMPERATURE TO THE GROWTH OF SWEET POTATOES

The control plants used in the above experiments have given some interesting data on the relation of temperature to growth of sweet potatoes. The soil moisture was the same at all temperatures, and in no case were any of the controls diseased. The difference in growth of the plants at the various temperatures can therefore safely be assumed to be due to the differences in temperature.

The plants were subjected to temperatures ranging from approximately 10° to 38° C. at 5° intervals up to 35°. At the lower temperatures the leaves turned a yellow, sickly color in three to four days, and gradually died. At 15° no growth took place, but the plants were able to hold their own with the exception of the occasional yellowing of some of the lower leaves. Above 15° the growth increased with the increase of temperature up to 35°. The growth at 38° was not quite equal to the growth at 35°, although it was very good. Judging from the results of these experiments the minimum temperature for growth is between 15° and 20°, the optimum at approximately 35°, and the maximum above 38°. It seems safe to predict that the maximum would be 40° or above.

A résumé of the data shows that sweet potatoes will grow at a temperature above which infection by the black-rot organism will take place. Likewise it is quite evident that infection will take place at a temperature too low to permit growth.

SUMMARY

Series of experiments have been conducted on the relation of soil temperature and soil moisture to infection of sweet potatoes by the black-rot fungus, *Ceratostomella fimbriata* (E. & H.) Ell. Some infection occurred at a temperature of 10° C., but none at 35° C. At temperatures between 15° and 30°, 100 per cent of the plants became diseased.

The optimum temperature for the disease was found to be about 25° C.

Soil moisture was found to exercise considerable influence on the amount of disease. The amount of disease increased with the increase in the percentage of the water-holding capacity of the soil up to about 60 per cent, and then decreased. There was some infection in a saturated soil. It was shown that infection took place at a moisture content of the soil as low as that at which the plant would grow. Based on percentage of infected plants, 100 per cent of the plants became diseased in soils with a water-holding capacity of 14 to 100 per cent.

Wounding is not necessary to infection, inasmuch as 48.2 per cent of the sprouts from potatoes dipped in a spore suspension of the black-rot organism and then bedded became diseased.

Observations on the growth of control plants showed that at 10° C. the plants slowly died, and that at 15° C. no growth took place, the plants merely holding their own. The optimum for growth appeared to be at about 35° C. There was a good growth at 38° C.

THE MOVING AVERAGE AS A BASIS FOR MEASURING CORRELATED VARIATION IN AGRONOMIC EXPERIMENTS [1]

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INTRODUCTION

In comparing varieties of cereals it is necessary to determine (1) which are more productive on a given soil in a given year and (2) which are more productive on this same soil over a period of years. Even this information is of value only for the particular soil type on which the experiments were conducted. To make the information of general application, the comparison must be repeated in different places to determine which varieties are more productive on soils of different types or levels of productivity. Each of these determinations is a problem in random sampling. Consequently, the use of the standard error, or its constant function, the probable error, is essential if the results are to be completely evaluated. Moreover, each element of the comparison is more or less distinct. A failure to obtain an accurate evaluation of a difference for a single soil and season can not be compensated for by repetition on different soils or in different seasons. It therefore becomes necessary to determine the probability that a difference in any individual experiment was or was not due to chance, and it is with this phase of the problem that the present paper primarily is concerned.[2]

In varietal experiments, the variation in yield of a variety on different plats may be divided into that due to variation in the sampling of (1) the variety, (2) the soil, and (3) all other conditions, which may be termed variation due to the experiment. The extent to which each of these is a factor is not known, but it generally is conceded that soil heterogeneity is many times as important as both of the other factors combined. The recognition of this importance led to the use of check or control plats, and more and more replications. The use of check plats constituted an effort to measure the extent of soil variation directly. The larger number of replications constitutes an effort to obtain a reliable mean without correcting for soil heterogeneity.

Check plats require land and labor that otherwise could be devoted to additional replications or varieties. Moreover, adjusting yields to checks involves an arbitrary assumption that may or may not be warranted in any given case. On the other hand, increasing the number of replications not only tends to make the experiment more reliable in itself but also reduces the probable error of the mean,

[1] Received for publication Nov. 11, 1925; issued June., 1926.

[2] Throughout the discussion, it is assumed that systematic errors due to competition, differences in stand, and the like were avoided by proper arrangement of the experiment, leaving only the variation due to random sampling to be measured. The probable error gives no information as to the cause of a difference but only the probability that it is significant, i. e., that it is, or is not, due to chance.

provided always that the probable error of a single result is not increased by extending the experiment onto other soil. Unfortunately, the number of replications usually is limited, and the reduction in the probable error of the mean decreases only as the square root of the number of replications increases. Moreover, in practice, increasing the area frequently increases the absolute variation in the experiment. The limit to the reduction in the probable error by replicating therefore soon is reached. Finally, no matter how much of the probable error of the mean may be eliminated by replicating, a large part of the remaining error will be due to soil heterogeneity. This part in no way lessens the accuracy of the results, yet a satisfactory way to eliminate it would be well worth while. With this in mind, the writer (*13*)[3] suggested regression on a moving average as a means of correcting for soil heterogeneity.

At about the same time "Student" (*3*) presented a formula for obtaining a generalized standard error for a difference between any two of m varieties in a varietal experiment. There were certain essentials in which this resembled the moving-average method. The two methods also differed fundamentally. It therefore seemed of interest to ascertain just what the differences were. This was done, and the relations found suggested a modification of the moving-average method. It is the purpose of the present paper to present this modification, together with certain simplifications of the moving-average method, and to consider the similarities and differences between it and the method presented by "Student." These similarities and differences depend largely upon the relations between the standard errors of the means of two variables, the standard error of their difference, and the coefficients of correlation and regression. Therefore, before taking up a detailed consideration of the two methods it is desirable to review certain aspects of the standard error of a difference.

THE STANDARD ERROR OF A DIFFERENCE

Using the convenient term "variance" which Fisher (*4*, *5*) has applied to the squared standard deviation (σ^2), the variance of a difference between two variables is given by the equation—

$$\sigma^2_{1-2} = \sigma^2_1 + \sigma^2_2 - 2r_{12}\sigma_1\sigma_2 \qquad (1)$$

If the two variables are uncorrelated, the last term on the right of this equation becomes zero, and the variance of the difference may be had from—

$$\sigma^2_{1-2} = \sigma^2_1 + \sigma^2_2 \qquad (2)$$

The value obtained by using equation 2 in any case is identical with that which would be obtained by taking the differences between the two series in every possible way and computing the variance of all such differences. Although equations 1 and 2 give identical results when $r=0$, they differ fundamentally in the philosophy that underlies them. The use of equation 1 implies that facts are known which suggest the possibility of correlation between the variables. The use of equation 2 may imply that there is no cor-

[3] Reference is made by number (italic) to "Literature cited," p. 1175.

relation between the variables, although it more usually implies that no reason is known for making comparisons between the series in any specific order.

An excellent hypothetical example of this difference may be had from an experiment with fruit trees. Assume that two treatments are compared, each treatment being applied to a group of 10 plats, the plats receiving treatment A not being adjacent to those receiving treatment B. Much of the variation among the plats of each treatment probably would be due to soil heterogeneity, the higher-yielding plats being on the more fertile soils, and *vice versa*. In the absence of more definite information there would be no basis on which to pair the plats in the order of their relative production and equation 2 would be used. If, on the other hand, data obtained on the yields of the same plats under uniform treatment prior to beginning the experiment had shown the inherent productivity of the plats to be in this (or any other) order, it would be very desirable to pair the plats as indicated by the preliminary data. Obtaining the variance of these differences would be equivalent in effect to using equation 1.[4] The same logic applies, of course, if the series of plats receiving the two treatments were known to be more comparable in any specific order because of juxtaposition or for any other reason.

It is rare that there is not some obvious reason for pairing certain plats in field experiments with cereals. Not to eliminate the correlated portion of the variance is to make the probable error larger and the results less conclusive than need be. Indeed, as has been pointed out (*12*), one objection that has been raised to the probable error by agronomists in the United States has been that differences which clearly were significant agronomically, frequently were not significant in terms of their probable errors. Much credit is due to Love for calling attention (*8, 9, 11*) to the fact that in these cases the direct computation of the probable error from successive differences could be used more properly, and so obtaining a wider usage of the pairing method. It is unfortunate, however, that Love has failed to distinguish sometimes between the effects of (1) pairing observations and (2) using Student's tables of probability (*1*) in applying what has been called "Student's method."

Thus, Love and Brunson (*12*) have used both the tables for the normal curve and for Student's curve in computing the probable significance of the differences in a number of experiments. In doing this, however, they have used the standard error computed by equation 2 in connection with the Gaussian curve and the standard error computed from successive differences with Student's curve. As there was more or less correlation between the series this really constituted a comparison between Student's probability tables applied to the correct standard error and the more usual tables applied to an incorrect standard error of the difference. Again, Love (*11, p. 88*) states, "The application of Student's method presupposes that experiments are so conducted that pairing may be accomplished."

The result has been to confuse the facts to an unfortunate degree. For example, Wiggans (*15, p. 6*) states that, "By the determination

[4] It should perhaps be noted that when only two items are being compared, equation 1 rarely will be used in practice, as the direct computation from successive differences is easier. The underlying theory, however, is the same.

of the probable error according to Bessel's or Peter's formula as an aid in interpreting the significance of a given lot of data, the consistency of differences in both amount and direction is often completely overshadowed by large seasonal variations. Seasonal differences, which enter so largely in the final expression of the determination of the probable error, affect very little the final results as determined by Student's method and expressed in odds." Again, it is stated in a recent publication (*7, p. 6*) that: "Bessel's method has been generally employed rather than Student's because the small number of plants used has made it possible to maintain similar soil conditions and because the comparisons averaged have been made, usually, in a single season, so that systematic errors were not important."

Inasmuch as Student's earlier contribution (*1*) primarily was the determination of probability values for use with small samples, rather than a method for pairing observations to eliminate correlated variation, the extent of the confusion is manifest. Thus, in the first example (*15*), Student's tables were better suited to the data than the ordinary ones because of the small number of seasons sampled. The standard error of the successive differences would have been the same, regardless of which tables were used, and the probability based on this standard error and the ordinary tables would have been unaffected by seasonal variation. In the second example (*7*), the standard error of the difference obtained with equation 2 is correct, but the use of Student's tables, or the more convenient ones published by Love (*10*) from which the odds may be read directly, would have been preferable because of the small number of plants used. In view of these facts, it is interesting that another of Student's early papers (*2*) considered correlation between adjacent plats, and that the method presented in his more recent paper (*3*) is essentially a short method for eliminating the average correlated among the yields in a varietal experiment.

NOTATION

For convenience, the symbols for the constants needed in the moving-average method and that presented by Student will be defined and illustrated in connection with a hypothetical experiment.

An experiment is assumed in which four varieties are replicated five times. To permit a continuous moving average without eliminating end plats, the plats are arranged in a circle. The assumed data are shown in Table 1.

The plat numbers in the first column of this table of course are arbitrary, as the plats have been assumed to be in a circle, with Nos. 1 and 20 adjacent. The plat yields are unimportant. The deviations from the means, in the fourth column, are the basis for all computations, except in the later illustration of Student's full 1923 formula. The assumed circular arrangement of plats permits using all deviations an equal number of times in computing the moving average, or index of productivity, shown in the fifth column. Thus, the I value of 2.0 units for plat 1 is the mean of the deviations of plats 19, 20, 1, 2, and 3. In the last column, the means of the deviations within each replication are the basis for Student's σ^2_σ when the experiment is considered as consisting of five replications or groups.

TABLE I.—*Assumed data showing method of (1) adjusting to the regression on a moving average, and (2) adjusting on the basis of replications*

Plat No. [1]	Variety	Assumed yield	*F* Deviation from varietal mean	*I* Moving average of deviations	*G* Mean of replicate deviations
1	2	3	4	5	6
1	A	24	4	2.0	
2	B	22	0	.4	−0.5
3	C	22	−2	−.8	
4	D	22	−4	−2.4	
5	A	18	−2	−3.2	
6	B	18	−4	−3.2	−3.0
7	C	20	−4	−3.2	
8	D	24	−2	−3.2	
9	A	16	−4	−2.0	
10	B	20	−2	−1.2	−1.0
11	C	26	2	−.8	
12	D	26	0	.4	
13	A	20	0	.8	
14	B	24	2	.8	
15	C	24	0	1.2	1.0
16	D	28	2	2.0	
17	A	22	2	2.4	
18	B	26	4	3.2	3.5
19	C	28	4	3.6	
20	D	30	4	3.2	

[1] Plots are assumed to be in a circle with Nos. 1 and 20 contiguous.

$m=4.0$	$s=5.0$	$n=5.0$
$\sigma^2_T=13.0$	$\sigma^2_I=5.184$	$\sigma^2_G=4.7$
$\sigma^2_E=5.0$	$r^2_{FI}=0.8226$	$r^2_{FG}=0.5875$
$\sigma^2_F=8.0$	Regr.$_{FI}=1.1575$	Regr.$_{FG}=1.000$

Using Student's notation, σ^2_T is the total variance of the experiment, i. e., the variance obtained when the deviation of each plat is taken from the grand mean without reference to variety or grouping. The variance of the group means is σ^2_G, and σ^2_E is the variance of the varietal or race means. Student further designates as σ^2_e "the casual error, which is the only part subject to random sampling" (*3, p. 282*).

The use of the deviations from the varietal means is necessary in connection with the moving-average method. These deviations, which may be considered as due to field variation apart from varietal influence, will be designated *F*. Their variance, σ^2_F may be computed either directly, or from the equation—

$$\sigma^2_F=\sigma^2_T-\sigma^2_E \qquad (3)$$

The correlation of *F* with *G* necessitates using one *G* value with each of the component plats to obtain the product moment, p_{FG}. Finally, the symbol p_{FF} indicates the product moment, when each *F* value is multiplied by each of the others in the same replication, and the sum of these is multiplied by $\frac{2}{mn(m-1)}$ to obtain the mean. In this terminology, $\frac{\sigma^2_{F-F}}{n-1}$ is the variance for the difference be-

tween the means of any two varieties, $(n-1)$ being used instead of n to correct for the small number, in keeping with Student's usage.

ELIMINATING GROUP VARIATION

The method presented by Student in 1923 (*3*) is essentially one for eliminating the average correlated variation among the varieties in the different replications from the average variance of all varieties in the experiment. The equation for accomplishing this, for the proof of which Student gives credit to R. A. Fisher, is—

$$\frac{\sigma^2_e}{n}=\frac{m(\sigma^2_T-\sigma^2_R-\sigma^2_G)}{(m-1)\ (n-1)}.$$

Twice this,

$$\frac{2\ \sigma^2_e}{n}=\frac{2m\ (\sigma^2_T-\sigma^2_R-\sigma^2_G)}{(m-1)\quad (n-1)}, \tag{4}$$

gives the average variance for the differences among the varieties in the experiment.

Student shows that the value for the variance of a comparison from this very simple formula is the same as that obtained by taking the successive differences between A and B, C, D, etc.; B and C, D, etc.; etc.; and averaging these $\frac{m\ (m-1)}{2}$ variances, a method that would be very laborious.

Using this equation with the values obtained from Table I,

$$\frac{2\sigma^2_e}{n}=\frac{2\times 4\ (13.0-5.0-4.7)}{3\times 4}=2.2$$

This is identical with the value obtained by computing the six variances for the successive differences between A, B, C, and D, averaging them, and dividing by $(n-1)$. Moreover, it is almost 50 per cent less than the variance for a difference obtained by equation 2, thus,

$$\frac{\sigma^2_{F-F}}{n-1}=\frac{8+8}{4}=4.0$$

It may be noted in passing that equation 4 may be used also to eliminate the correlated variation due to seasonal fluctuation from the average standard error for the differences among several varieties or treatments compared in a number of seasons. In such a comparison σ^2_G becomes the variance of the seasonal means. As the final value obtained is the mean of the variances for all possible comparisons, care should be used to include only data that are reasonably comparable. Thus, if one of the treatments might be expected to vary much more widely than the others with differences in seasonal conditions, the use of this equation scarcely would be warranted. It should be borne in mind, moreover, that the variance based upon the deviations of the annual mean values measures only the variation due to fluctuation in soil and climate from year to year. The true variance should measure also the variation within the individual experiments.

REGRESSION ON A MOVING AVERAGE

The method previously suggested by the writer (*13*) for adjusting for soil heterogeneity consisted in (1) expressing each plat yield as a percentage of the mean yield of the variety grown in that plat, (2) computing a moving average based on a group of several contiguous plats, (3) determining the regression of the individual plats on the moving average, and (4) adjusting the individual plat yields on the basis of this regression and the deviations of the moving average.

In using this method it became evident that it was unnecessarily complicated. The use of deviations of the individual plat yields from the mean yields of the varieties grown in them obviated the computation of percentage yields. It also was unnecessary to adjust each individual yield. The corrected variance may be had by multiplying the gross variance by $(1-r^2_{FI})$, where r_{FI} represents the correlation between the individual and the moving-average deviations,or, symbolizing the variance of F for constant I by ${}_I\sigma^2_F$,

$$ {}_I\sigma^2_F = \sigma^2_F (1-r^2_{FI}) \tag{5} $$

Finally, the mean of each variety may be adjusted to the mean of all moving-average values for that variety. This eliminates further computation, and usually gives essentially the same final result. Even if varieties differing extremely in yield were included in the experiment, it is questionable whether a corrected error based on percentage deviations would be more reliable than one based on actual deviations.

So much for these changes which are but simplifications. The real modification, suggested by Student's (*3*) presentation, is the inclusion of a further term, $\frac{s}{s-1}$, in equation 5. The final equation for obtaining the variance of a mean difference, after adjusting to the regression on a moving average, then is—

$$ \frac{2\,s\,\sigma^2_F(1-r^2_{FI})}{(s-1)(n-1)}, \tag{6} $$

where s is the number of plats used in computing the moving average. The reason for including the term $\frac{s}{s-1}$ will be discussed later. It is desirable first to apply equation 6 to the data in Table I,

$$ \frac{2\times5\times8\,(1-0.8226)}{4\times4}=0.8870 $$

The variance for a difference between the means of any two of the varieties in the hypothetical experiment shown in Table I, therefore, would be 0.8870 after the yields were adjusted to a moving average of 5 adjacent plats. This is materially smaller than the variance of 2.2, obtained by equation 4; and that of 4.0 obtained by equation 2. Accordingly, it is preferable if it can be justified.

COMPARISON OF METHODS

As previously noted, there are certain fundamental similarities and differences between the methods. One important difference was eliminated by introducing the term $\frac{s}{s-1}$ into the equation for correcting the variance by the moving-average method. The effect of this term is the same as that of $\frac{m}{m-1}$ in equation 4, namely, to compensate for the fact that the individual plat deviations are components of the averages with which their correlation was determined and therefore that the coefficient obtained is partly the result of the correlation of the individual deviations with themselves. This may be shown most easily through equation 1.

Modifying equation 1 so that it applies to the variance of a difference between any two of the m variables,

$$\sigma^2_{F-F} = \sigma^2_F + \sigma^2_F - 2r_{FF}\sigma_F\sigma_F = 2(\sigma^2_F - p_{FF}). \tag{7}$$

But, as the means of the deviations of the plats in the replications constitute the deviations of the replications, $\sigma^2_G = p_{FG}$, and

$$p_{FF} = \frac{m\,\sigma^2_G - \sigma^2_F}{m-1}.$$

Substituting the latter equality for p_{FF} in equation 7,

$$\sigma^2_{F-F} = 2\left(\sigma^2_F - \frac{m\,\sigma^2_G - \sigma^2_F}{m-1}\right).$$

or, simplified and with $\frac{1}{n-1}$ introduced to obtain the variance for a mean difference,

$$\frac{\sigma^2_{F-F}}{n-1} = \frac{2m(\sigma^2_F - \sigma^2_G)}{(m-1)(n-1)}. \tag{8}$$

In view of the equality in equation 3, this is equivalent to equation 4.

With the terms $\frac{m}{m-1}$ and $\frac{s}{s-1}$ included, the only essential difference between the methods is that one is based on the regression on a moving average, whereas the other is based on the regression on the means of the replications. Thus, the variance of F for constant G is given by the equation—

$$_G\sigma^2_F = \sigma^2_F(1 - r^2_{FG}), \tag{9}$$

which is analagous to equation 5.

But,

$$r^2_{FG} = \frac{p^2_{FG}}{\sigma^2_F\,\sigma^2_G},$$

and

$$p_{FG} = \sigma^2_G.$$

Therefore,

$$r^2_{FG} = \frac{\sigma^2_G}{\sigma^2_F}.$$

Substituting this in equation 9, and simplifying,

$$_{o}\sigma^2_{\bar{x}} = \sigma^2_{\bar{x}} - \sigma^2_{a}, \tag{10}$$

which, with the term $\frac{m}{m-1}$ included, and multiplied by $\frac{2}{n-1}$ to give the variance of a mean difference, gives a result identical with that obtained by equations 4 or 8.

Both methods, therefore, consist essentially in obtaining the average variance of the individual plats for a constant group value, and using this in place of the total variance in determining the standard error of a difference.

So much for the resemblance between the methods. The question then is whether the replication average or the moving average is a better basis for measuring the variation due to soil. It seems reasonably clear that the moving average is better in any case where the number of plats used in computing the moving average approximates the total number of strains being compared. In the hypothetical case under consideration, for example, strain A always occurs adjacent to strain D in the previous replication, whereas it is two plats removed from strain D in the same replication. With a 5-plat moving average, the corrected variance for A is based on its correlation with B in the same replication, with C in the same and in the previous replications, and with D in the previous replication. The corrected variances for the other sorts are on a corresponding basis. That is, the correlated variation for which correction is made with the moving-average method is that between plats of one strain with the nearest plats of the other strains, rather than with the plats of the other strains occurring in the same more or less arbitrary replication groups.

In order to compare the methods in an actual experiment, the moving-average method has been applied to the data presented by Student (*3*). These are the yields of individual plats of barley in Beaven's No. 1 yield experiment of 1913. The data comprise a comparison of eight strains of barley compared in square plats arranged in chessboard fashion in a strip of 5 plats wide by 32 plats long. Each plat was formed by sowing eight rows 4 feet long and 6 inches apart, the seed being spaced 2 inches apart in the row. One row on each side and 6 inches at each end of the other rows of each plat were rejected at harvest to avoid the effects of competition. This left the yields of plats 1 yard square as the basis of comparison. The yields and planting order are shown in Table II, being the same as shown by Student in Diagram 1 (*3, p. 277*). The mean yields of the strains were computed first (Table IV, column 2), and the deviation of each plat from the mean of the strain grown in it was obtained (Table II, columns 3). A moving average of the deviations of 4 or 5 adjacent plats then was computed. For plats 2 to 31 inclusive in Series II, III, and IV, the 5 deviations averaged were those of a central plat with the plats to the north, south, east, and west of it. The same principle was followed around the perimeter of the field, but only four plats were available for averaging. The average value for a corner plat was the mean deviation of the four plats in that corner. The moving-average values, or indexes of productiveness, for all plats are shown in Table II, columns 4.

TABLE II.—*The yields, deviations from the means, and indexes, in grams, for 160 plats of barley grown in Beaven's No. 1 yield experiment of 1913* [1]

Plat No.	Series I				Series II				Series III				Series IV				Series V			
	Strain	Yield	Deviation	Index	Strain	Yield	Deviation	Index	Strain	Yield	Deviation	Index	Strain	Yield	Deviation	Index	Strain	Yield	Deviation	Index
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	A	236.5	−61.6	−57.6	B	265.9	−34.8	−54.4	C	265.6	−53.1	−48.5	D	255.9	−39.4	−61.1	E	230.1	−76.3	−60.7
2	F	210.4	−66.1	−55.8	G	236.7	−67.9	−47.0	H	205.0	−66.8	−64.6	A	222.6	−75.5	−58.2	B	249.3	−51.4	−48.9
3	C	291.1	−27.6	−35.3	D	295.8	+0.5	−42.9	E	246.7	−59.7	−41.4	F	218.7	−57.8	−49.2	G	312.2	+7.6	−33.4
4	H	223.9	−47.9	−36.5	A	238.3	−59.8	−28.4	B	277.4	−23.3	−55.1	C	258.2	−60.5	−34.4	D	263.4	−31.9	−29.9
5	E	295.6	−10.8	−20.1	F	265.0	−11.5	−30.4	G	232.3	−72.3	−23.0	H	273.3	+1.5	−40.8	A	263.4	−34.7	−17.1
6	B	290.5	−10.2	−9.3	C	320.9	+2.2	−5.5	D	286.0	−9.3	−25.9	E	268.2	−38.2	−9.7	F	273.4	−3.1	−20.0
7	G	286.1	−18.5	−14.2	H	272.9	+1.1	−7.0	A	286.1	−12.0	−13.4	B	301.1	+0.4	−14.5	C	314.9	−3.8	−20.1
8	D	266.2	−29.1	−15.5	E	298.7	−7.7	−15.1	F	229.3	−47.2	−36.7	G	285.7	−18.9	−44.6	H	198.0	−73.8	−43.8
9	A	291.6	−6.5	−14.4	B	307.9	+7.2	−21.6	C	221.2	−97.5	−64.4	D	212.0	−83.3	−55.9	E	227.9	−78.5	−77.7
10	F	247.3	−29.2	−9.1	G	301.3	−3.3	−35.5	H	170.7	−101.1	−55.6	A	296.7	−1.4	−59.9	B	225.7	−75.0	−48.5
11	C	321.4	+2.7	−24.9	D	244.2	−51.1	−32.3	E	231.8	−74.6	−66.2	F	237.7	−38.8	−32.8	G	265.4	−39.2	−61.3
12	H	249.9	−21.9	−18.5	A	263.1	−35.0	−38.4	B	235.5	−65.2	−41.0	C	308.8	−9.9	−50.6	D	203.0	−92.3	−50.7
13	E	286.5	−19.9	−28.7	F	257.5	−19.0	−18.9	G	284.4	−20.2	−41.4	H	225.0	−46.8	−30.8	A	236.9	−61.2	−62.2
14	B	246.6	−54.1	−19.5	C	318.2	−0.5	−13.9	D	239.7	−55.6	−17.6	E	290.4	−16.0	−34.0	F	228.0	−48.5	−37.7
15	G	301.0	−3.6	+10.0	H	331.7	+59.9	+15.5	A	302.2	+4.1	+1.5	B	297.5	−3.2	−2.0	C	293.8	−24.9	−27.7
16	D	333.0	+37.7	+18.9	E	324.0	+17.6	+23.7	F	278.8	+2.3	+7.3	G	334.4	+29.8	−3.6	H	237.6	−4.2	+0.2
17	A	322.0	+23.9	+17.1	B	301.7	+1.0	+9.4	C	301.2	−17.5	−7.7	D	282.6	−12.7	+21.2	E	336.6	+30.2	+3.2
18	F	282.4	+5.9	+33.2	G	326.8	+22.2	+9.6	H	260.3	−11.5	+18.2	A	374.3	+76.2	+21.1	B	330.2	+29.5	+38.7
19	C	399.3	+80.6	+43.9	D	325.5	+30.2	+27.3	E	328.0	+21.6	+17.5	F	300.4	+23.9	+40.0	G	323.5	+18.9	+29.5
20	H	330.8	+59.0	+29.8	A	280.2	−17.9	+26.6	B	324.1	+23.4	+17.5	C	377.9	+59.2	+36.7	D	341.0	+45.7	+38.2
21	E	303.9	−2.5	+34.4	F	315.0	+38.5	+1.3	G	305.9	+1.3	+21.6	H	303.0	+31.2	+33.1	A	327.0	+28.9	+34.4
22	B	343.3	+42.6	+2.0	C	305.9	−12.8	+15.8	D	308.7	+13.4	−0.6	E	351.5	+45.1	+33.1	F	308.1	+31.6	+28.0
23	G	285.2	−19.4	−0.3	H	269.2	−2.6	−3.0	A	248.0	−50.1	+0.2	B	344.7	+44.0	+11.7	C	324.9	+6.2	+27.2
24	D	273.5	−21.8	+16.0	E	376.4	+70.0	+11.1	F	272.7	−3.8	+6.9	G	317.7	+13.1	+29.5	H	298.7	+26.9	+18.7
25	A	333.4	+35.3	+11.6	B	314.2	+13.5	+39.1	C	324.2	+5.5	+21.2	D	362.4	+67.1	+29.6	E	334.8	+28.4	+34.6
26	F	295.9	+19.4	+42.5	G	376.0	+71.4	+52.2	H	295.6	+23.8	+16.3	A	331.8	+33.7	+32.5	B	316.6	+15.9	+7.9
27	C	362.6	+43.9	+68.6	D	428.4	+133.1	+60.6	E	253.5	−52.9	+22.4	F	298.7	+22.2	+0.9	G	258.2	−46.4	−1.0
28	H	349.8	+78.0	+79.6	A	405.7	+107.6	+78.2	B	286.7	−14.0	+31.3	C	366.4	+47.7	+22.8	D	299.8	+4.5	+11.7
29	E	395.3	+88.9	+88.3	F	362.9	+86.4	+82.4	G	372.6	+68.0	+44.2	H	325.6	+53.8	+56.2	A	338.9	+40.8	+35.9
30	B	400.6	+99.9	+77.6	C	379.9	+61.2	+70.3	D	322.3	+27.0	+58.3	E	377.0	+70.6	+50.0	F	321.0	+44.5	+38.6
31	G	364.9	+60.3	+75.9	H	348.6	+76.8	+65.6	A	362.9	+64.8	+54.5	B	354.7	+54.0	+41.0	C	317.2	−1.5	+23.0
32	D	361.8	+66.5	+67.1	E	371.3	+64.9	+64.5	F	326.3	+49.8	+49.2	G	321.8	+17.2	+29.0	H	266.8	−5.0	+16.2

[1] Yield data and arrangement taken from Student, on testing varieties of cereals (*3, p. 277*).

The coefficient of correlation between the deviations of the individual plats and the indexes was determined from the correlation table (Table III). The average variance for a single plat, and for the mean of 20 plats, was computed from the same table.

TABLE III.—*Correlation table for the deviations in yield (in grams) of individual plots of barley, and the indexes for such plots computed as a moving average for values in columns 3 and columns 4 of Table II*

Class centers	INDEXES																	Total
	−75	−65	−55	−45	−35	−25	−15	−05	+05	+15	+25	+35	+45	+55	+65	+75	+85	
INDIVIDUAL PLATS																		
−105			1															1
−95		1	1															2
−85			1															1
−75	1	2	1	2		1												7
−65		2	2	2	1													7
−55				4	1	1	2		1		1							10
−45					4			1										5
−35		2	1		2	1	1	1	1									9
−25			1	1	1	1	2	1		1								8
−15				1	2	2	3	3		2	2	1						16
−05			2		1	2	4	2	2	1	1	1						16
+05				2	1	2	1	2	3	1	3	1						16
+15								1	1		4	1	1					8
+25								1	2	5		4		1				13
+35									2	2	2	2						8
+45									1	1	1	4	1		1			9
+55										1	1	1	1	1				5
+65										1	1		1	1	2	2		8
+75											1		1	1	1	1		5
+85													1				2	3
+95																1		1
+105																1		1
+115																		0
+125																		0
+135																1		1
Total	1	7	10	12	13	10	13	12	13	15	17	15	6	4	5	5	2	160

Individual plats: $\sigma_F = 46.165$ gm, $\sigma^2_F = 2{,}131.25$ gm. Indexes: $\sigma^2_I = 38.389$ gm, $\sigma_I = 1{,}473.73$ gm. $r = 0.8386$, $r^2 = 0.7032$, $(1-r^2) = 0.2968$. Regression of individual plats on indexes = 1.009.

Letting F represent the deviations of the individual plats, and I represent the moving-average values as before, the multiplication of the gross mean variance for the 20 plats of a strain by $\frac{s}{s-1}(1-r^2_{FI})$, or $\frac{4.5}{3.5}(0.2968)$,[5] gives the corrected variance. The corrected variances obtained in this way are shown in column 4 of Table IV. The sum of the variances for any two strains gives the variance for a difference between them. If only the average corrected variance is desired, it may be obtained directly from the average gross variance. Thus,

$$112.2 \times \frac{4.5}{3.5}(0.2968) = 42.8,$$

the average variance for any strain mean. Twice this, 85.6, is the variance for a difference between any two of the strain means. The square root of this (9.25 gms.) times 0.6745 gives 6.2 gms. as the average probable error for a difference, or about 2.1 per cent of the

[5] s is taken as 4.5, the unweighted average of the 4 and 5 plats used in computing the moving average.

mean yield. This is materially lower than the error for a difference of 3.4 per cent based upon the gross variance of 112.2 gms. It also is 12.5 per cent less than the error of 2.4 per cent reported by Student (*3*), obtained by the equivalent of the pairing method.

TABLE IV.—*The mean yield in grams, the variance of the mean before and after adjusting to its regression on a moving average, and the correction for regression and for position, for eight strains of barley*

Variety	Mean yield [1]	Gross variance [1]	Variance after adjusting [2]	Correction for position [3]	Correction for regression [4]	Corrected yields on regression basis
1	2	3	4	5	6	7
A. English Archer	298.1	130.8	49.9	+2.3	+0.625	298.7
B. 145	300.7	95.0	36.3	+1.7	+2.125	302.8
C. 145/146	318.7	96.6	36.9	+1.0	+.125	318.8
D. Plumage	295.3	153.4	58.5	+.3	+.125	295.4
E. Early Archer	306.4	133.9	51.1	−.3	−1.375	305.0
F. Irish Archer	276.5	76.0	29.0	−1.0	−2.375	274.1
G. 7 A	304.6	80.2	30.6	−1.7	+2.125	306.7
H. Biffin	271.8	131.3	50.1	−2.3	−1.375	270.4
Average	296.5	112.2	42.8	0	0	296.5

[1] The means and variances in columns 2 and 3 differ slightly from those shown by Student (*3*), due to the fact that the means were computed directly without grouping. As all other computations made in this paper were based upon deviations from these means, similar discrepancies occur elsewhere.

[2] Raw variance multiplied by $\frac{4.5}{3.5}$ (0.2968), or 0.3816.

[3] Student's values, although their use was not recommended by him.

[4] Computed from class values shown in Table III, and corrected for mean of all index values, which was 0.125.

This difference is due largely to the fact that the correlated variation removed by grouping according to replication is not as great as when the moving average forms the basis. Thus, the plats of A always occur at some distance from the plats of D within replications. There are however 19 possible comparisons between A and D in adjacent plats, i. e., A in one replication with D in the preceding replication. When the comparisons are made in these two ways, the results are:

Comparison within replications A–D = 2.8 ± 7.82.

Comparison of adjacent plats A–D = 9.6 ± 7.15.

The variation in the mean difference is due to the exclusion of A in Series I, plat 1 from the comparison of adjacent plots. The reduction in the probable error is 8.6 per cent for this comparison, as compared with 12.5 per cent for the experiment as a whole. Strains A and D were among the most variable in the experiment. Adjusting the means and variances of A and D on the moving-average basis gives a difference of 3.3 ± 7.02. This difference is more in accord with that obtained when all plats are averaged, while the variance approximates that obtained by comparing only adjacent plats.

If the yield of each plat is adjusted to its index, the mean yield as well as the variance may be modified. As already noted, however, it is unnecessary in practice to adjust the yields of the individual plats, as the mean yield of each strain may be adjusted to the mean of the indexes for that strain. Thus, the means of the indexes for each strain in Beaven's experiment, with the signs changed, are shown

in column 6 of Table IV. The regression of F on I is given by $r_{FI}\frac{\sigma_F}{\sigma_I}=1.009$, so that the means may be corrected directly as indicated by the heading of column 6, "Correction for regression." The mean of the deviations of any strain of course is 0.[6] The mean of the indexes for a strain, therefore, is unaffected by the fact that the deviations of that strain were components of the indexes. Consequently no term comparable to $\frac{s}{s-1}$ has been used in the regression. Student (*3*) presented values for correction for position based on the facts that the plats became more productive toward one side, and that the varieties occurred in the same relation throughout. Student's values are shown in column 5 of Table IV. It is only fair to note, however, that Student did not recommend such correction for position. The correction values obtained in the two ways are in agreement as to sign except for strain G. The corrections for regression fail strikingly to present the orderly change from the maximum minus to the maximum plus of the other method. None of the corrections are important. Nevertheless, the mean index values furnish additional evidence on the accuracy of the experiment that is of some value. They would have been more reliable, however, if the order of the varieties had been changed in the different replications.

CONCLUSION

Yields obtained in comparisons of varieties or strains of cereals are relative, not absolute. They fluctuate from plat to plat, and from season to season, as a result of variation in soil, climate, plants, and other conditions of the experiment. Granted that gross or systematic errors have been avoided, the reliance that can be placed in the data depends upon the adequacy of random sampling. The best measure of this is the standard error, or its constant function, the probable error. From this may be derived a quantitative expression of the probability that a given result is, or is not, due to chance. The probable error, therefore, becomes a valuable tool in agronomic experiments. Unfortunately, however, it has certain limitations, and it has been misapplied and misinterpreted so frequently in the past as to make some investigators question the desirability of using it at all.

A frequent fault in using the probable error has been to draw conclusions that the odds were greatly in favor of a certain treatment or variety being superior, when as a matter of fact the probable error gave no information as to this. Thus, for example, the individual plants in two rows have been noted for some character, the probable error for the mean of each row and for the difference between the two means computed, and the conclusion drawn, in terms of this probable error, that there was a significant difference between the varieties or strains grown in the two rows. Evidently no conclusion is warranted as to the cause of the difference in such a case. It may have been due to soil, or seed, or both. The blame for a wrong conclusion, however, rests on the investigator making it rather than

[6] This is not strictly true in the present case as the influence on the index of any given plat in the perimeter is one-fourth its value, whereas in the body of the experiment it is only one-fifth.

on the probable error. Fortunately, such misuse of the probable error is becoming less with a better understanding of its real functioning.

It is with two other difficulties, however, that the present paper is concerned. One of these is in the fact that the limitations of land and labor frequently prevent enough replications of any one comparison to permit a strict statistical interpretation of the standard error in terms of probability. The method of computing a generalized error for the experiment, based on the average coefficient of variation, as suggested by Hayes (*6*), or on the average variance, as shown by Student (*3*) and used by the writer (*14*) and others, helps to overcome this difficulty. The other difficulty is that the standard error is a measure of the gross or total variation. In most field experiments a certain amount of the variation is concomitant. The productivity of the soil does not change abruptly but more or less gradually, so that adjacent plats are more nearly alike than plats that are farther apart. In general, moreover, varieties tend to respond similarly to these differences in soil productivity, as well as to climatic changes. Not to eliminate correlated variation from the standard error before using it to determine probability is to make the results seem less conclusive than they really are.

Where only two items are involved, pairing observations is a simple, direct way of eliminating correlated variation. When several items are being compared, however, this becomes unwieldy in the extreme. The method presented by Student (*3*) provides a quick, satisfactory means for eliminating the average correlated variation and providing a generalized error for the experiment. When many strains are being compared, as frequently is the case, the replication groups are large, the intergroup correlations are likely to be small, and correlated variation within these groups, of which no account is taken in this method, becomes more important. This seems to the writer to be a serious drawback. The moving average is a more flexible measure, the number of plats used in the average being changeable to meet the conditions of the experiment. It provides, furthermore, an index of the average productivity of the soil on which each strain was grown, whether this be used for adjusting yields or not. Even with few strains in the experiment, it would seem that the moving average provides a better basis for measuring correlated variation than the replication average. The difference would be slight, however, and probably would be more than offset by the greater convenience of the other method. On the other hand, Student's method (*3*) is a straightforward statistical process involving no assumptions whatever. This is true of the moving-average method only when the number of plats used in computing the moving average equals or exceeds the number of strains in the experiment. In other cases, the use of the moving average assumes that such an average gives a fair index of the productivity from plat to plat. This assumption seems entirely justified. The use of the moving average as a basis for eliminating correlated variation consequently seems safe and logical.

The principle involved in the method presented by Student (*3*) would seem to have a very important application in interpreting experiments repeated over a number of seasons. Here the group-

ing is determined arbitrarily, and the correlations are likely to be large. The use of equation 4 or equation 8, with G representing the seasonal means, should do much to eliminate some of the present cumbersome comparisons by pairs.

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FURTHER OBSERVATIONS ON THE RELATIONS BETWEEN GROWTH AND SAP CONCENTRATION IN CITRUS TREES[1]

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This paper reports on a continuation of studies on the correlation between sap concentration and the effects of pruning which have been reported in earlier papers (*6*, *7*)[2] from this laboratory.

RELATION BETWEEN SAP CONCENTRATION AND RATE OF GROWTH OF LEMON BRANCHES

The data here reported were obtained from Eureka lemon trees (*Citrus limonium* var.) growing in the orchard of the Citrus Experiment Station at Riverside, Calif. At the time the observations were begun the trees were 5 years old, having grown 2 years in the nursery and 3 years in the orchard. The trees were rather heavily pruned on March 17, 1920. Soon after growth started from the terminal region of the branches 76 lateral shoots were selected on 28 individual trees in different parts of the orchard. Four of these shoots had to be eliminated before the measurements were finished, leaving 72 shoots on which measurements were recorded from April 26 until December 20, 1920. On the day of the last measurement the shoots had a mean length of 139.17 ± 3.41 cm. In treating these data, it is assumed that they are more or less typical of the vegetative activity of young trees of this species.

INFLUENCE OF EXTERNAL FACTORS ON THE GROWTH OF LEMON SHOOTS

The writers shall first present some data on aspects of the growth of this lot of trees during 1920. Figures 1 and 2 show graphically the relation of growth to air temperature, to soil moisture, and to the rate of water loss from porous clay atmometers of the Livingston type between April 19 and December 20, 1920, in the grove mentioned. The graphs clearly show that there was no great amount of correspondence between the mean temperature and the mean increments in growth of the lemon shoots.

The curve of weekly increments in length of the shoots shows that the first part of the season was the time of greatest growth, and that there were two distinct cycles of growth, during the first of which these shoots made about five-sixths of all the growth of the entire season.

Until some agency intervenes to check their linear extension, lemon shoots are notably deficient in the production of secondary laterals, nearly all of their growth being confined to the elongation of the main axis. The records show that the average time of appearance of

[1] Received for publication Oct. 14, 1925; issued June, 1926, as Paper No. 136, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] Reference is made by number (italic) to "Literature cited," p. 1194.

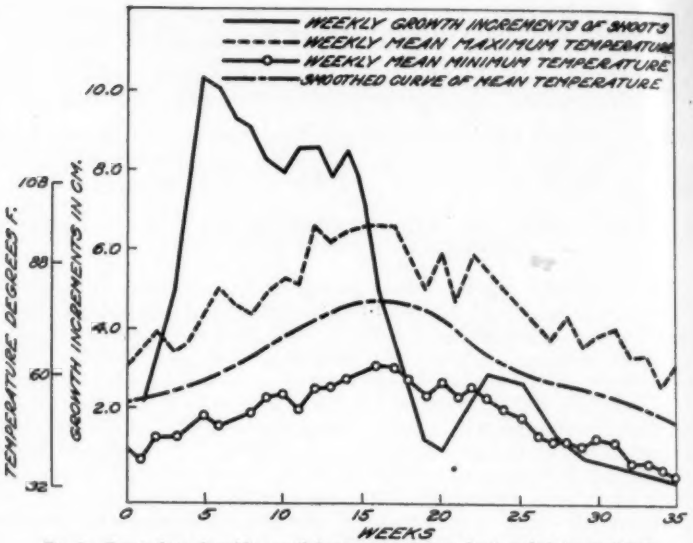

FIG. 1.—Comparison of weekly growth increments of lemon shoots and air temperatures

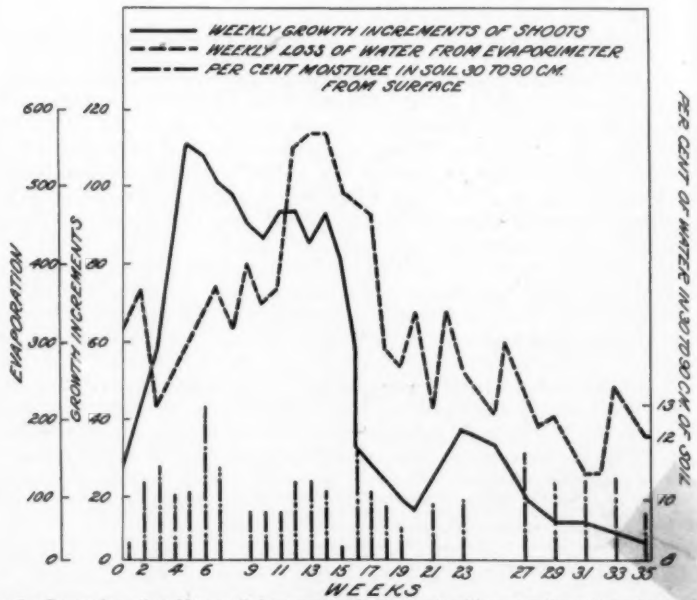
WEEKLY GROWTH INCREMENTS OF SHOOTS
WEEKLY LOSS OF WATER FROM EVAPORIMETER
PER CENT MOISTURE IN SOIL 30 TO 90 CM. FROM SURFACE
EVAPORATION
GROWTH INCREMENTS
PER CENT OF WATER IN 30 TO 90 CM. OF SOIL
WEEKS

FIG. 2.—Comparison of weekly growth increments of lemon shoots with evaporation rates and percentage of water in the soil

secondary laterals was 23.9 weeks after April 19 (approximately October 1). In practically every case these laterals appeared from one to three weeks after the second cycle of growth started, and were produced a short distance below the apex of the shoot. When the growing season ended, the laterals were only a few centimeters long, and of little significance as far as the total mass of material produced during the season was concerned. Flowers appeared on nine of the shoots between October 11 and November 22.

Since the water relations of the trees are so obviously connected with their growth, the writers have made observations on the water content of the soil in which the trees grew, as well as on the evaporating power of the air. During the period over which these growth records were made the soil received 22.57 acre-inches of irrigation water and a rainfall of 3.39 acre-inches, giving a total of 25.96 inches. The per cent of water in the soil at a depth between 30 and 90 cm. is shown by the vertical broken lines in Figure 2.

Although there was some fluctuation in the water content of the soil, which at times approached the wilting point, it is difficult to conclude that there is any causal relation between the rate of growth and the soil moisture in this case. Furthermore, there is no obvious correspondence between the evaporation rate and the growth rate. The former is unimodal, and the latter is distinctly bimodal.

WATER DEFICIT OF SHOOTS

The problem of water deficit in citrus trees growing in a region where the evaporation rate is high has been studied extensively by Bartholomew (*1*) and its importance indicated. The method the writers employed for the study of this factor was as follows: From shoots of uniform age, pieces 15 cm. in length were cut under water. The pieces were cut from the apical region because it was thereby possible to get more nearly comparable material. As soon as cut, the pieces were dried gently with a towel and weighed. They were then placed in a beaker containing enough tap water to cover the bases of the stems for a distance of 1 to 2 cm. and were immediately placed under a bell jar at room temperature for 24 hours. The atmosphere in the bell jar was soon saturated with water vapor and the shoots reached what might be termed their water-absorption capacity. At the end of the 24-hour period the shoots were removed from the beaker, dried with a towel, and again weighed. The increase in weight is ascribed to water which they had absorbed and is regarded as an index of their water deficit when taken from the shoot.

The water deficit thus determined varied considerably during the season. It increased rapidly from May until the first of July, reached its highest point near the first of August, and then rapidly declined to a level near that reached during June (fig. 3). There is a certain correspondence between the graphs of water deficit of shoots, soil moisture, and mean daily evaporation from porous clay atmometers. They indicate that the water deficit increased as the soil moisture decreased and the evaporation rate increased. The fluctuations in water deficit lag somewhat behind those of soil-moisture content and antedate those of the evaporation rate.

SEASONAL FLUCTUATIONS IN THE SAP CONCENTRATION OF LEMON SHOOTS OF UNPRUNED TREES

The methods of collecting the samples, killing the tissues by preliminary freezing, grinding, and pressing the material, were substantially the same as those described in a previous paper (7). The material consisted of the shoot and adhering leaves of the last obtainable cycle of growth. The cycles of growth on citrus trees are so clearly marked and have such definite physiological characteristics that it seems logical to make them the basis of selection of material.

The term "fruit wood" will be used in the following paragraphs to designate the shorter, slow-growing lateral branches which have a tendency to produce flowers and fruit. The term "shoot wood" designates the distinctly vegetative growth which arose from the terminal portion of various branches and grew rapidly with but little inclination to produce lateral branches during the first season. All

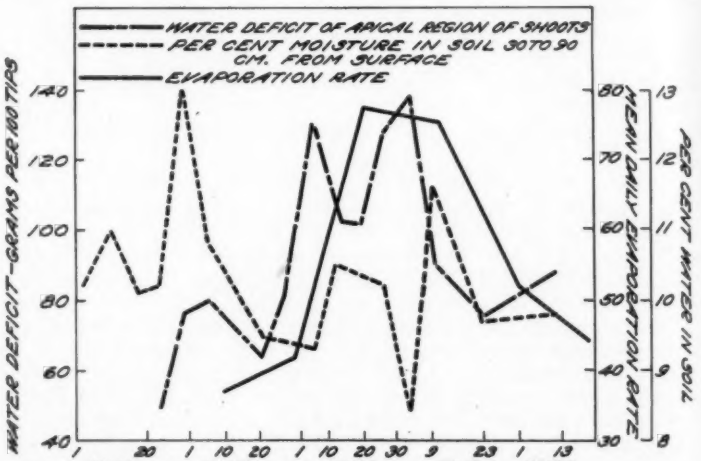

FIG. 3.—Comparison of water deficit of lemon shoots with soil moisture and evaporation rate

flowers and fruit were removed from the shoots, as their sap was more dilute than that of shoot and leaf.

The data on the concentration of the sap of lemon trees are given in Table I, together with records of irrigation, soil moisture, evaporating power of the air, and air temperature. Each plot contained five trees.

The graphs in Figure 4 show the variation in sap concentration of the fruit wood and the shoot wood during the year following May 5, 1920. The graphs were constructed from the data for plot 5 in Table I.

The concentration of the sap of the fruit wood was characteristically higher than that of the shoot wood. The maximum concentration was attained during the early winter and the minimum during the summer. The graph shows fluctuations, but the general trend of the values is unmistakable. The period of most active flowering on these trees came in March, before vegetative growth was active.

TABLE I.—*Concentration and P_H values of the sap of lemon trees*

Date	Soil moisture 30 to 90 cm.	Water added, in last 21 days	Mean daily evaporation	Mean daily temperature of last 21 days	Plot	Osmotic pressure of sap		P_H of sap	
						Fruit wood	Shoot wood	Fruit wood	Shoot wood
	Per cent	*Acre-inches*	*C. c.*	*° F.*		*Atm.*	*Atm.*		
1920									
Feb. 11	9.0	[a] 1.80		55.5	4	18.83	19.16	5.85	5.93
Mar. 3	10.8	[a] 2.41		53.5	8	16.10	16.88	6.14	6.10
Mar. 25	11.0	[a] 2.66		54.8	10	19.28	17.15	5.75	5.97
Apr. 14	10.3	[a] .51		56.0	13	18.48	14.92	5.14	5.34
May 5	11.3	[b] 1.22		58.2	4	20.34	12.86	5.28	5.68
					5	20.44	17.48	5.14	5.31
May 26	10.2	[a] .68		64.0	4	17.51	14.15	5.19	5.16
					5	19.46	15.74	5.12	5.16
June 9	12.3	[b] 1.20	37	69.2	4	16.74	13.88	5.22	5.33
					5	16.72	14.93	5.16	5.22
					8	16.64	14.21	5.17	5.44
					10		14.42		5.34
					13	16.97	12.86	5.14	5.53
June 30	10.5	[b] 1.35	42	70.4	4	16.17		5.26	
					5	17.08	14.02	5.19	5.31
					8	17.16	14.27	5.24	5.44
					10	16.96	14.15	5.21	5.44
					13	16.61	13.61	5.19	5.61
July 21	10.3	[b] 1.30	77	79.2	4	16.37	14.79		
					5	14.98	15.05		
					8	16.29	14.74		
					10	15.50	14.99		
					13	15.53	14.42		
Aug. 11	11.5	[b] 4.10	75	82.7	4	14.96	14.52		
					5	15.52	15.99		
					8	15.17	15.84		
					10	15.72	14.57		
					13	15.10	14.72		
Sept. 1	9.1	.00	52	77.9	4	16.58	15.84		
					5	17.56	15.21		
					8	16.60	14.94		
					10	17.18	16.96		
					13	17.07	16.13		
Sept. 21		[b] 2.50	44	75.5	4	16.06	14.44	5.17	
					5	17.64	15.82	5.31	
					8	16.58	14.33	5.00	
					10	16.24	14.90	5.41	
					13	16.01	14.74	5.65	
Oct. 14		[b] 4.00	40	69.0	4	16.66	16.16	5.38	5.97
					5	16.62	16.26	5.31	5.48
					8	17.04	14.85	5.43	5.85
					10	16.68	16.30	5.44	5.93
Nov. 4	10.3	[b] 4.80		61.6	4	18.45	19.24	5.39	5.65
					5	21.27	15.03	5.36	5.99
					8	20.07	17.94	5.61	5.65
					10	19.85	17.03	5.31	5.82
					13	18.10	17.30	5.55	5.53
Nov. 23	10.4	[b] 2.10		59.3	4	19.62	15.16	5.48	6.15
					5	19.19	18.18	5.63	6.04
					8	18.36	16.19	5.53	5.83
					10	19.49	17.51	5.53	6.04
Dec. 16	9.2	[a] .31		53.8	4	20.96	14.98	5.61	6.02
					5	21.34	16.96	5.61	6.07
					8	20.67	17.80	5.48	6.02
					10	20.24	17.98	5.61	5.95
					13	19.91	17.63	5.61	5.93
1921									
Mar. 10		[a] .35		59.4	4	20.16	16.74	5.65	6.04
					5	20.02	15.72	5.82	5.85
					8	20.20	16.13	5.80	6.05
					10	20.04	16.76	5.75	6.00
					13	19.12	16.55	5.75	6.05
Mar. 31		[a] 2.11		58.0	4	19.76	15.17	5.78	5.93
					5	18.53	15.77	5.70	5.95
					8	18.44	15.96	5.75	5.88
					10	19.85	15.17	5.60	5.85
Apr. 20		[b] 5.40		54.1	4	19.19	15.23	5.83	6.00
					5	19.54	16.47	5.82	5.88
					8	12.05	16.08	5.82	5.99
					10	18.81	16.24	5.77	5.83

[a] Irrigation. [b] Rainfall.

Much of the young fruit resulting from the March blossoms fell in the latter part of May. The trees continued to bloom, but the percentage of fruit lost by abscission was very large. The fruit wood made a cycle of fairly active growth late in June and early in July, and it seems logical to attribute the minimum sap concentration observed during that time to its active vegetative condition. A third cycle of growth set in about the middle of October, accompanied by the appearance of the fall crop of flowers. No data on the cyclic distribution of fruit buds are given here, but the subject was rather fully presented in a paper (*6*) published seven years ago, in which it was shown that flowers appeared chiefly in the spring and fall.

Neglecting minor fluctuations in the graph for the present it may be said in a general way that blossoms were produced chiefly at a time when the sap concentration of the fruit wood was at or near the

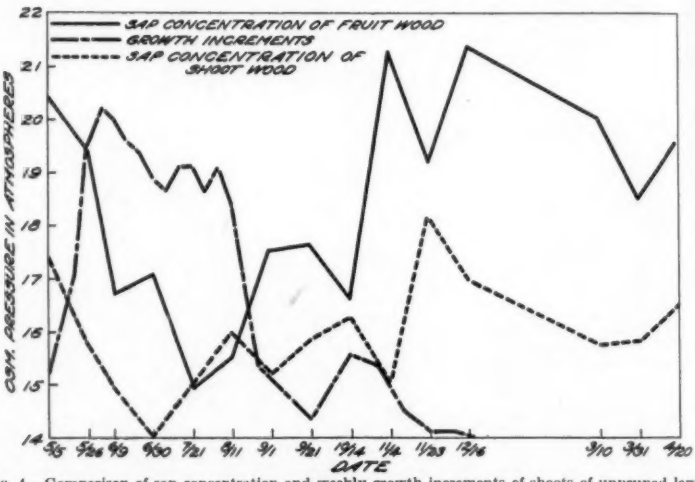

FIG. 4.—Comparison of sap concentration and weekly growth increments of shoots of unpruned lemon trees

maximum, and that vegetative growth was produced when the sap concentration was at or near the minimum.

The sap concentration of the shoot wood shows a seasonal cycle which, however, is not quite as clearly marked as that of the fruit wood. The lowest concentration was observed in the latter part of June, and was followed by a rise through two rather distinct steps to a maximum in November. It may be useful to compare the weekly increment in length of vegetative shoots with the graph representing sap concentrations. Maximum growth and minimum sap concentration have a correspondence somewhat similar to that reported for apricot trees (*7*). It may be assumed that the rapid rate of metabolism incident to the periods of most rapid growth is characterized by the presence of a condition in which the plant fluids are more dilute and have a greater tendency to rapid diffusion.

RELATION OF SAP CONCENTRATION OF LEMON TREES TO PRUNING

TIME OF PRUNING

The trees in four plots were rather severely pruned at different times in the spring of 1920, and their sap concentration was studied during the remainder of the period ending April 20, 1921. These plots are adjacent to plot 5 (the unpruned control), and the data may be compared with those already presented. The trees of plots 4, 8, 10, and 13 were pruned on February 11, March 3, March 25, and April 14, respectively. On the day when each plot was pruned, samples of fruit wood and shoot wood were collected and sap concentrations determined (Table I). On May 5 the new shoots on trees in plot 4 were advanced far enough for sampling. On and after June 9 shoots were taken from all the plots, usually at intervals of three week.

Examination of the data in Table I, to see whether the pruning had any significant effect on sap concentration, shows that with two exceptions the sap of the shoot wood from plot 4 was generally more dilute than that from plot 5 until the end of the following December. The trend of the differences in the case of the fruit wood is not so well defined as to be apparent on casual inspection. On May 26 the sap of fruit wood from plot 5 was materially higher than that from plot 4, which had been heavily pruned. The sap of trees from plot 5 was more dilute on July 21 than that of any of the other plots.

The mean differences for the entire period between the pruned trees and the unpruned trees is shown in Table IV. In this work the writers have calculated the odds that the differences are significant from Love's modification (*5*) of Student's table. For statistical reliability there should be odds of at least 30:1 that the differences are significant, *i. e.*, are not due to chance variation of the material or of the technic. Plot 13 was the only plot showing an undoubted dilution in the sap of the trees in comparison with the control, although the chances are fair that the sap of the trees in plot 4 was really less concentrated than that of the control. The writers should say that there is no difference in the case of trees in plots 8 and 10.

In the case of shoot wood, where the concentration of sap was appreciably less than in the fruit wood, the differences are not so well marked, at least not in plots 10 and 13. The differences in plots 4 and 8 are positive, but their statistical reliability is quite doubtful.

The study was carried further the following year with older (8-year-old) lemon trees bearing a good crop of fruit. The data are shown in Table II.

Four plots, each containing six trees, were selected on the Lemona Heights grove, near Riverside, Calif. The trees were moderately pruned by a system which consists in thinning and heading back the young upright growth. Plots 16, 17, and 18 were pruned May 11, July 13, and September 29, respectively; plot 19 served as a control and was not pruned during the period of these observations. Samples consisting of not fewer than 50 shoots were taken periodically from each plot, and the concentration of the sap was ascertained by the freezing-point depression.

TABLE II.—*Concentration and PH values of sap of 8-year-old lemon trees*

Date	Soil moisture 12 to 36 inches	Plot	Osmotic pressure of sap		PH of sap	
			Fruit wood	Shoot wood	Fruit wood	Shoot wood
1921	*Per cent*		*Atm.*	*Atm.*		
May 11	6.8	[a] 16	18.35	.....	5.65	.....
		17	17.61	.....	5.77	.....
		18	17.74	.....	5.71	.....
		19	17.86	.....	5.71	.....
June 1	8.4	16	18.56	.....	5.82	.....
		17	17.79	.....	5.75	.....
		18	17.98	.....	5.77	.....
		19	17.66	.....	5.82	.....
June 22	8.6	16	13.85	13.28	6.15	5.97
		17	13.94	.....	6.12	.....
		18	13.89	.....	6.02	.....
		19	14.28	.....	6.12	.....
July 13	8.2	16	15.26	16.23	5.88	6.07
		[a] 17	15.29	.....	6.14	.....
		18	15.38	.....	6.02	.....
		19	14.94	.....	6.15	.....
Aug. 3	6.0	16	14.55	15.89	.....	.....
		17	14.66	15.92	.....	.....
		18	14.16	.....	.....	.....
		19	14.36	.....	.....	.....
Sept. 29	.....	16	16.13	14.79	5.78	5.93
		17	15.11	14.24	5.85	5.85
		[a] 18	15.18	.....	5.65	.....
		19	15.22	.....	5.71	.....
Nov. 10	.....	16	15.47	16.46	5.48	5.85
		17	16.18	16.28	5.77	6.07
		18	15.96	12.82	5.77	5.48
		19	16.47	.....	5.71	.....
Dec. 15	.....	16	16.97	.....	5.40	.....
		17	16.17	.....	5.80	.....
		18	16.72	.....	5.70	.....
		19	16.76	.....	5.40	.....

[a] Trees pruned at this time.

The trees made good growth from May to November. On June 22 the average length of the shoot wood produced as a response to pruning on May 11 was 20 cm. On July 13 the production of the first set of blossoms had mostly ceased, in fact none was to be found on the trees in plot 16 (pruned May 11), although the shoot wood taken from that plot in sampling was about 70 cm. long. On August 3 the fruit wood was still devoid of blossoms and otherwise in a quiescent condition. The shoot wood from plot 17 (pruned July 13) was growing rapidly and had reached a length of approximately 20 cm. A new cycle of growth was under way on September 29 which resulted in the production of blossoms on the fruit wood and in increased elongation of the shoot wood. On November 10 the vegetative shoots on plots 16, 17, and 18 had average lengths of 175 cm., 80 cm., and 12 cm., respectively. The differences are due in part to the difference in the time at which the trees were pruned, and in part to the resting stage which appeared in the latter part of the summer. The growth of the trees differed in no significant manner from that of other healthy lemon trees in this region.

The data given in Table II make it extremely doubtful, however, whether pruning had any marked effect on the sap concentration of the fruit wood of the trees. Reference to Table IV shows that the mean differences through the season were small, and that the odds that they are statistically significant are very small indeed. Particular attention was paid to the concentrations of sap from the fruit

wood at the determination immediately following the pruning of the trees, but, as the figures show, there was no apparent dilution in this case as a result of pruning. The concentration of the sap of shoot wood was less frequently determined and there is only one case (plot 18) in which there appears to have been a pronounced drop in concentration following pruning.

The writers present the results of these determinations in this rather extensive fashion because they feel that it is necessary to establish the conclusions which they support. The senior author thought at one time that he had evidence that pruning lemon trees severely does lower the sap concentration of the fruit wood. In the light of the more abundant evidence now at hand the writers are inclined to conclude that pruning lowers the sap concentration only in very young trees.

An interesting comparison afforded by a single case was obtained May 25, 1921, from the sap of young lemon trees, some of which had been pruned the preceding October, and had been thereby stimulated to active growth. The concentration of the sap of the fruit wood from pruned and unpruned trees was 14.80 and 18.70 atmospheres, respectively. One occasionally finds differences as great as this, but over a long period appreciable differences are not as common as is sometimes assumed.

SEVERITY OF PRUNING

An additional series of determinations was made on the sap of young lemon trees, comparing the effects of severe and moderate pruning. The trees grew in the Citrus Experiment Station orchard, and were 3 years old at the beginning of the observations reported here. Plots 20 and 21 (Table III) were severely pruned; plots 22, 23, and 24 were lightly pruned; and plot 25 was not pruned. In 1920 the trees were pruned April 20, and in 1921 on March 18. On the former date the trees had a heavy crop of blossoms, but in the succeeding months most of the young fruit developed therefrom fell to the ground. On July 20, 1920, the trees in plots 20 and 21 received a small amount of additional pruning. The fruit was weighed when picked on July 23, 1920, and at each picking thereafter. In 1920 (four pickings) the average weight of fruit per tree, except for the unpruned trees, was small on account of the age of the trees. In 1921 the average weight of fruit per tree was as follows: Unpruned, 48.3 pounds (21.9 kg.); lightly pruned, 47.9 pounds (21 7 kg.); and severely pruned, 23.9 pounds (10.8 kg.). In the last case the reaction to the severe pruning produced an active vegetative growth which could not produce fruit in the time under discussion.

The various determinations of the sap concentration of the several plots of trees are given in Table III. They show somewhat the same seasonal fluctuations as those already mentioned. The differences between severe and moderate pruning are not, as a rule, very conspicuous, although there are individual cases in which sensible differences occurred. From the data in Table IV it appears that in three cases the sap of fruit wood was more concentrated, and in two cases more dilute, than that of the fruit wood of the unpruned trees. The sap of trees of plot 22 was slightly but significantly more concentrated than that of the unpruned trees. In the other cases the differences were negligible.

TABLE III.—*Concentration and P_H values of sap from lemon trees which had different pruning treatments*

Date	Plot	Osmotic pressure of sap		P_H of sap		Date	Plot	Osmotic pressure of sap		P_H of sap	
		Fruit wood	Shoot wood	Fruit wood	Shoot wood			Fruit wood	Shoot wood	Fruit wood	Shoot wood
1920		*Atm.*	*Atm.*			1921		*Atm.*	*Atm.*		
June 3	20	16.79	.....	.....	.....	Apr. 28	24	20.27	13.94	5.75	6.14
	21	16.54	.....	.....	.....		25	19.46	14.75	5.63	6.10
	22	17.42	.....	.....	.....	June 8	20	15.09	.....	5.77	.....
	25	17.30	.....	.....	.....		21	14.79	.....	5.99	.....
Sept. 29	20	16.36	14.30	.....	.....		22	15.45	.....	5.95	.....
	21	16.31	15.14	.....	.....		23	15.41	.....	5.82	.....
	22	17.73	16.08	.....	.....		24	15.40	.....	5.99	.....
	23	16.86	15.96	.....	.....		25	15.41	.....	5.82	.....
	24	16.50	15.15	.....	.....	July 20	20	14.40	.....	6.04	.....
	25	16.72	15.92	.....	.....		21	14.98	.....	5.99	.....
Dec. 1	20	16.79	14.94	5.61	5.95		22	15.35	.....	6.10	.....
	21	16.91	16.04	5.61	5.87		23	.....	.....	.....	.....
	22	16.46	15.56	.....	.....		24	14.73	.....	6.05	.....
	23	16.65	15.33	.....	.....		25	15.00	.....	5.82	.....
	24	.....	.....	.....	.....	Oct. 4	20	14.28	.....	5.75	.....
	25	17.87	16.76	5.90	5.87		21	13.64	.....	5.70	.....
1921							22	14.04	.....	5.65	.....
Feb. 3	20	20.12	18.03	5.80	6.07		23	13.40	.....	5.71	.....
	21	19.61	18.84	5.65	6.27		24	13.64	.....	5.75	.....
	22	20.90	18.28	5.53	5.95		25	12.88	.....	5.75	.....
	23	20.49	17.86	5.77	6.07	Nov. 17	20	16.91	.....	5.80	.....
	24	20.31	18.57	5.83	6.05		21	15.71	.....	5.80	.....
	25	20.04	17.67	5.78	6.10		22	15.90	.....	5.80	.....
Mar. 17	20	20.18	16.17	5.85	5.99		23	15.89	.....	5.80	.....
	21	19.74	17.22	5.83	5.93		24	15.72	.....	5.80	.....
	22	19.52	18.34	5.93	6.04		25	15.90	.....	5.70	.....
	23	18.17	16.74	5.63	5.99	1922					
	24	18.64	17.90	5.90	5.99	Mar. 16	20	18.60	.....	.....	.....
	25	18.81	16.19	5.70	5.99		21	18.69	.....	.....	.....
Apr. 28	20	19.84	13.70	5.78	6.09		22	18.75	.....	.....	.....
	21	18.94	13.66	5.83	6.14		23	.....	.....	.....	.....
	22	19.73	13.94	5.82	6.19		24	18.86	.....	.....	.....
	23	19.85	15.14	5.75	6.15		25	18.78	.....	.....	.....

The sap of the shoot wood of the trees in plot 20 was materially lower in concentration than that of the control trees, although the odds that the difference is significant (Table IV) are only 15 : 1. In the case of the other plots there is no significant mean difference in sap concentrations.

TABLE IV.—*Mean differences between concentration of sap of pruned lemon trees and their unpruned controls*

[Differences expressed in atmospheres of osmotic pressure. Odds that the differences are significant calculated by Student's method]

Plot	Fruit wood	Odds	Shoot wood	Odds	Plot	Fruit wood	Odds	Shoot wood	Odds
	Atm.		*Atm.*			*Atm.*		*Atm.*	
4	+0.43	21.0 : 1	+0.75	9.4 : 1	18	+0.07	2.5 : 1		
8	+.21	5.5 : 1	+.16	7.4 : 1	20	−.11	2.0 : 1	+0.83	15.0 : 1
10	+.15	2.9 : 1	−.12	2.2 : 1	21	+.21	6.4 : 1	+.08	[b]
13	+.86	39.0 : 1	+.09	[a]	22	−.28	31.9 : 1	−.18	1.6 : 1
16	−.20	3.9 : 1	.....	.....	23	+.05	[c]	+.05	1.8 : 1
17	+.10	3.7 : 1	.....	.....	24	−.12	3.9 : 1	−.26	1.8 : 1

[a] Less than 1 : 1. [b] Less than 1.3 : 1. [c] Less than 1.5 : 1.

Summarizing the data, it appears that neither severe nor moderate pruning affects the sap concentration of leaves and shoots of lemon trees for more than a few weeks after the operation. This does not eliminate the possibility that the temporary differences may pro-

foundly affect the physiological activities of the trees, if they occur at critical periods of fruit-bud differentiation, fertilization of the flower, or of other processes.

When the effect of pruning upon sap concentration of lemon trees is compared with that of apricot trees (*7*), a contrast is readily seen. In the latter case there was a consistent difference throughout the season. The writers have made some computations from the data in Table III of the article published in 1921 (*7*), partly for their own reassurance and partly because it represents the activity of a tree of different growth habits. It was found that the mean difference in sap concentration based upon 21 determinations between April 16 and October 8 was 2.02 atmospheres of osmotic pressure, and that the odds that the difference was significant are as infinity is to 1. In view of these results, the writers seem justified in concluding that there is something about the lemon tree which operates to maintain osmotic pressure in the face of a growth rate undergoing rather extreme fluctuations.

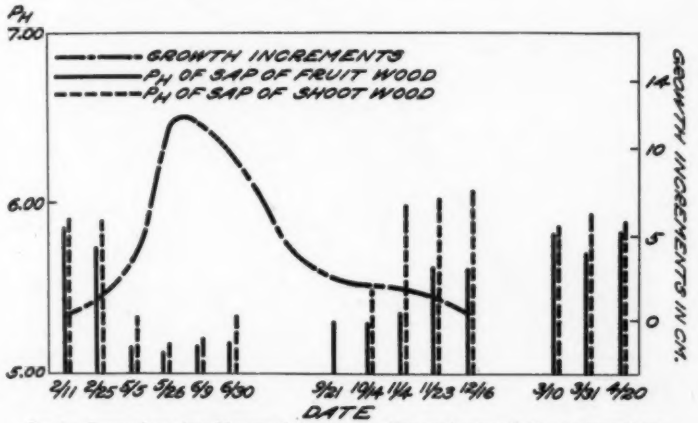

FIG. 5.—Comparison of weekly growth increments of lemon shoots with P_H of expressed sap

HYDROGEN-ION CONCENTRATION OF SAP FROM LEMON LEAVES AND SHOOTS

A portion of each sample taken for the determinations shown in Tables I to III was used for an electrometric determination of the hydrogen-ion concentration.[3]

The data for the P_H of the expressed sap as presented in Figure 5 show a certain, though small, relationship to the growth of the trees concerned. Slightly increased acidity is found in the samples during the time of more rapid growth of the trees. The range is between P_H 5.0 and P_H 6.0, and the slight differences should not be emphasized, perhaps, except for the fact that they show a seasonal variation. The existence of an analogous seasonal variation in the acid content of apple fruit spurs has been reported by Hooker (*3*).

The sap from fruit wood seems to be slightly lower in P_H than that from the shoot wood of the same trees. So far as the effects of

[3] The writers are indebted to their colleague, A. R. C. Haas, for these determinations.

pruning on the P_H of the sap are concerned, no significant differences can be seen. The relative constancy of the P_H of the sap may be due to correlated processes which maintain the reaction, or to the presence of buffers which produce the same result.

The titration curves of expressed sap from fruit wood and shoot wood are decidedly similar in nature (fig. 6), though that of fruit wood probably has more buffer power.

Contemporary studies in this laboratory (*8*) have shown that the reaction of the medium surrounding the roots of citrus trees has no

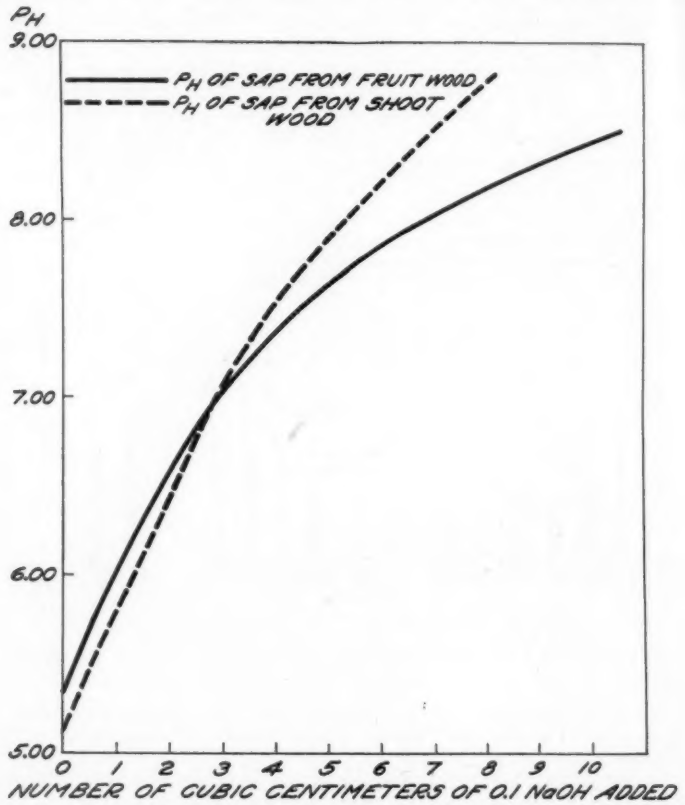

FIG. 6.—Titration curves of expressed sap of lemon trees

material influence upon the reaction of the sap of their leaves (*8*, *Tables 11*, *16*), although Haas (*2*) found the contrary relation in the case of certain annual plants.

So far as the writers have been able to learn from the literature on this subject, the reaction of the sap of a plant tends to remain uniform, except in cases where the cells are injured. However, the writers recognize the limitations of their experimental methods. It is probable that the sap obtained by grinding and pressing does not

exactly represent the contents of the cells, so, therefore, any statement may need qualification. Nevertheless, it is believed that the unextracted substances would probably exert more buffer action rather than less, and that there is perhaps a greater constancy of reaction than the writers' data indicate. It is possible that the slight increase in acidity during the season of rapid growth was due to the temporary lower concentration of buffer substances in the leaf cells.

INFLUENCE OF SEASON AND CULTURAL OPERATIONS ON SAP CONCENTRATION OF ORANGE TREES

In 1921 one of the writers (7) published some data on the sap concentration of vegetative shoots of the orange tree, in which it was shown that the concentration was least in May and June and then gradually increased to October, when the observations ceased.

The former observations will now be extended. Fruit wood of the orange (*Citrus sinensis*) was used instead of shoot wood as a source

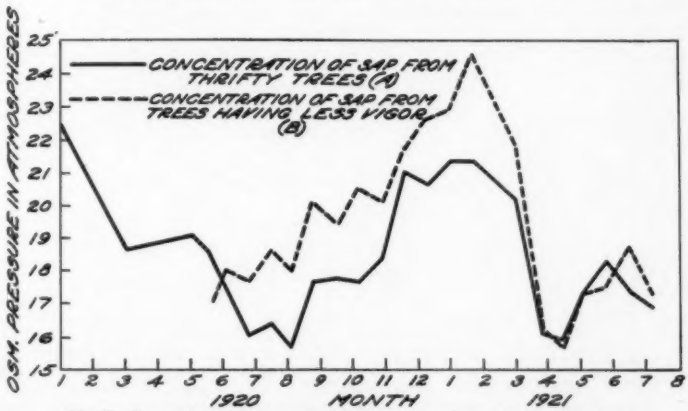

FIG. 7.—Comparison of the concentration of sap from orange trees in two groves

of material. The graphs in Figure 7, representing the seasonal changes in sap concentration, show somewhat the same trend as that for lemon fruit wood, reaching maxima at the close of the season of most active growth and exhibiting minima during the season of active growth.

The data were obtained from trees in two orange groves selected on account of their contrasts. A represents a grove of navel orange trees on Hanford sandy loam soil near Highlands, Calif., in which the trees are more than 30 years old, extremely thrifty, and productive. B represents a 20-year-old navel orange grove on Ramona loam near Riverside, Calif., in which the trees are distinctly less thrifty and productive.

Between February 1 and April 14, 1920, the rainfall at grove A was 12.36 inches, and at grove B 8.43 inches.

The soil conditions in grove B are less favorable than in the former, owing both to the nature of the soil and to the cultural system. This soil becomes increasingly impervious to irrigation water

as the summer advances, and the soil moisture in parts of the root zone is below the wilting point during a considerable portion of the growing season.

Trees in grove A were in full bloom April 9, 1920, and the amount of bloom was not excessive. On May 18 the fruits were approximately 0.7 to 1 cm. in diameter, and very few showed indications of abscission. The trees in grove B usually produce numerous blossoms and set many fruits which suffer severely from the premature abscission commonly called "June drop," although many of the blossoms are shed before the perianth opens. The period of blossoming in the B grove coincided with that in grove A. On May 19 the young fruit in grove B was approximately equal in size to that of grove A, but the former suffered very extensive abscission, which continued throughout practically all of June and was followed by a second period of fruit abscission in August. The mature fruit in the two cases was decidedly different. That of grove A was large and

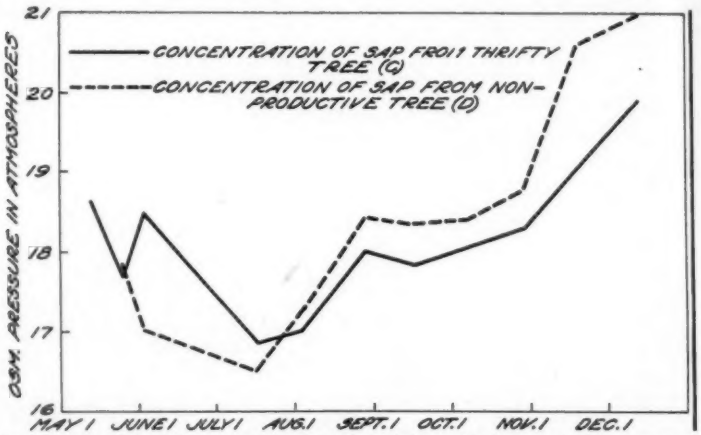

FIG. 8.—Comparison of the concentration of sap from thrifty and from nonproductive orange trees in experiment plots

of good quality; but that of grove B was small with many split open at the distal end. In 1921 there was again a heavy abscission of fruit about June 15.

Graphs representing the concentration of the sap of fruit wood in A for a period of 20 months and in B for 15 months are shown in Figure 7. The character of the seasonal variation is rather similar in the two cases, but the range of B is greater than that of A. The mean difference in sap concentration in the fruit wood in groves A and B from May 19, 1920, to April 1, 1921, was 1.89 atmospheres of osmotic pressure. The season of minimum concentration was found to coincide, as in other instances, with that of rapid vegetative growth. In grove A in 1920, the period of lowest sap concentration was in July and August, at a time when the sap concentration of the wood in grove B had made a definite rise. In 1921, however, both lots of trees seemed to reach minimum sap concentration simultaneously.

During the year 1920 determinations were also made of the sap concentration of the fruit wood of two orange trees which, although not widely separated, behaved very differently with respect to the

June drop and other features. These trees are located on the Rubidoux tract of the Citrus Experiment Station, and have been closely observed for several years. The tree which is designated C was comparable to those of lot A, previously described, *i. e.*, it was thrifty and lost comparatively little fruit from the June drop. The tree designated D was unthrifty and lost an excessive amount of fruit during the June drop, and may be compared with the trees of lot B already described.

The concentration of the sap of the fruit wood of these trees is shown by the graphs in Figure 8. In one respect these graphs resemble those for groves A and B already discussed, viz, the sap of the unthrifty tree was more dilute than that of the thrifty tree during the late spring and early summer, but more concentrated in the autumn and winter. The mean difference in sap concentration in trees C and D was only 0.11 atmosphere, because the plus and minus differences tended to cancel each other, although, if the algebraic signs of the differences be disregarded, the average differences would be 0.59 atmosphere.

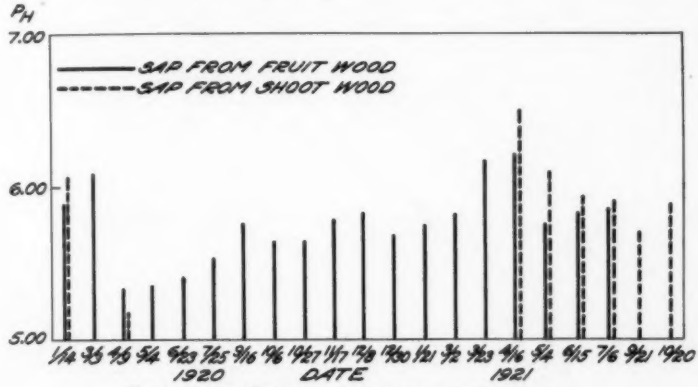

FIG. 9.—Seasonal fluctuations in the P_H of sap of orange trees

SEASONAL CHANGES IN THE HYDROGEN-ION CONCENTRATION OF SAP FROM ORANGE TREES

The sap expressed from the fruit wood of the orange trees was used for making determinations of the P_H. The results agree with those for the lemon trees previously presented in showing relatively little variation during the time of the observations. Figure 9 shows the relative P_H values of sap of trees in grove A through a period of nearly two years. The P_H value was lowest during April and May, 1920, but was high during March and April of the following year. This speaks against any strong belief in a seasonal change in acidity of the expressible portion of the sap. The maximum range, however, was from P_H 5.34 to P_H 6.22. The acid content of apple fruit spurs (*3*) has been found to reach a maximum during the period of most active growth concomitantly with a minimum carbohydrate concentration.

The acid reserve of the sap is shown by Figure 10, which represents the titration curves of sap from both fruit and shoot wood. They show the apparent lack of any strong buffer action.

These results are confirmed by a majority of the workers on this question. On the whole, the hydrogen-ion concentration of the expressed sap of a given plant is influenced relatively little by the usual range of fluctuations of external conditions, though Kelley and Cummins (*4*) found that the acidity of orange leaves increased with their age. In the present work relatively young leaves were dealt with each time, and the acid content remained fairly constant.

THE FRACTION OF SOLUBLE MATERIAL REMOVED BY PRESSING LEAF MEAL

The question is frequently asked, "How representative of the cell contents is this liquid pressed from the plant tissues?" The obvious

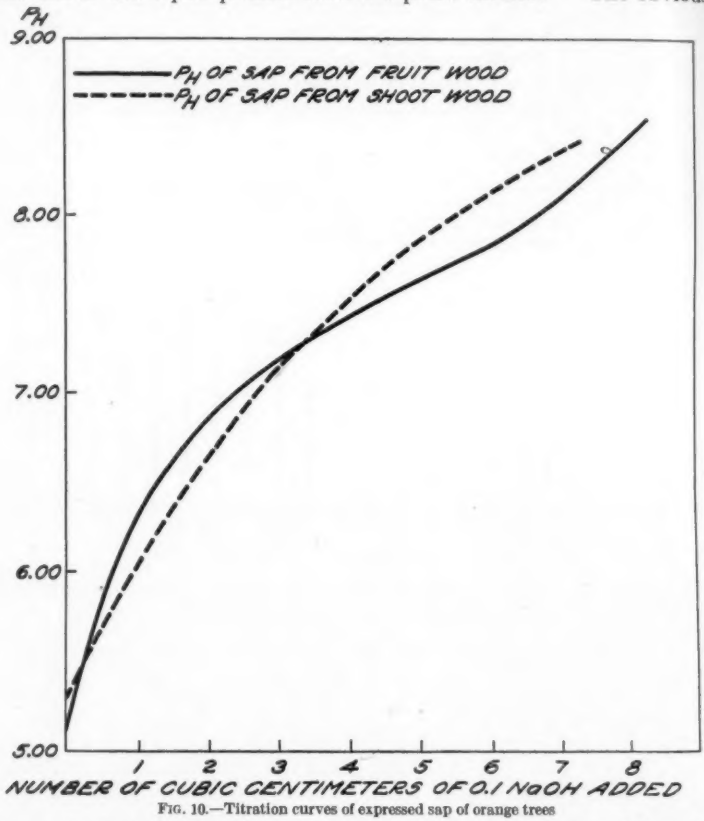

FIG. 10.—Titration curves of expressed sap of orange trees

answer is that the sample is not strictly representative, and that the degree of correlation between the original and the expressed sap will vary with the nature of the plant tissues employed. The writers have undertaken some determinations of the concentration of successive portions from a sample of orange-leaf meal.

A sample of the ground leaf tissue was pressed in a powerful screw press until increasing applications of pressure yielded no more sap. The effluent sap was measured, then the press was opened enough to

permit the addition of distilled water to the moist press cake. The quantity of water added was exactly equal to the volume of sap pressed out. The press cake was not removed from the cloth, as it was possible to add the entire quantity of water slowly from a pipette. After the water had had time enough for distribution through the press cake, the press was screwed down and another quantity of liquid collected, equal in volume to the first sample. This was repeated until the concentration was greatly reduced. The later samples were not nearly as deeply colored as the first, and were more quickly obtained from the press cake. The indexes of refraction of the samples were determined, and from them the percentages of soluble matter were obtained.

Three series of determinations are presented herewith (Table V). The orange sap samples designated A were from mature leaves of thrifty young trees, and those designated B were taken late in the season from young Valencia orange trees in an inadequately irrigated grove. The leaves were collected from trees in grove B which had survived a severe hot wind which had caused damage to much of the foliage. To this fact the writers are inclined to ascribe the rather high content of soluble matter. This sample was rich in viscous materials, and the second, third, and fourth extractions were not very widely different in concentration. The determination of sap concentration in walnut-leaf sap is an average of two sets of determinations made on sap from trees grown under experimental conditions. The successive decreases in concentration are somewhat similar to those of the later extractions of orange leaves in the A sample.

TABLE V.—*Analyses of the sap extracted in successive extractions from leaf meal, showing the percentage of soluble substances in each fraction*

Leaf meal of—	Fraction						
	I	II	III	IV	V	VI	VII
Orange A	25.7	12.2	7.3	5.0	3.5	------	------
Orange B	35.1	15.3	12.7	10.0	7.8	5.7	4.8
Walnut	15.2	6.6	4.6	3.0	------	------	------

These figures indicate that the amount of solutes unextracted by the first application of pressure may be large, but there seems to be no reasonable doubt that the first extraction yielded a truly representative sample. The writers do not feel that one is justified in summing the percentages obtained in this way and regarding the sum as the amount of soluble material in the plant sap. It would be necessary to evaporate to dryness and weigh the collected residues.

SUMMARY

The seasonal fluctuation in the sap concentration of citrus trees bears a certain inverse proportionality to their rate of growth, the highest concentration having been found during periods of more or less complete dormancy, and the lowest during periods of active vegetative growth. Although neither growth nor sap concentration bore any intimate relation to the accompanying fluctuations in temperature, evaporation, or soil moisture, yet there was a certain correspondence between the evaporating power of the air and the water deficit of lemon twigs, although the variations of the latter usually lagged somewhat behind the former.

The concentration of sap obtained from fruit wood of citrus trees was generally higher than that from shoot wood of similar age. Blossoms appeared on the lemon trees at periods which corresponded fairly well with the time at which the sap concentration of the fruit wood reached a maximum.

The sap concentration of lemon trees often showed some depression as a result of severe pruning, but usually this effect was transient. Repeated comparisons over an entire season do not show differences of sap concentration of such magnitude or reliability as to lend support to the contention that pruning greatly alters the concentration of the sap, except possibly for brief periods. However, one must recognize the possibility that a temporary depression of sap concentration might affect the activity of the tree if it occurred at a critical physiological period.

Determinations of the sap concentration of thrifty and of unthrifty orange trees showed rather significant differences. While the fruit was setting, the thrifty trees had a higher sap concentration; but as the summer passed the unthrifty trees suffered from scanty irrigation and other untoward cultural conditions, with the result that their growth was retarded and the sap concentration was raised. The thrifty trees suffered much less from June drop than the unthrifty trees.

The hydrogen-ion concentration of the sap of both lemon and orange trees shows a rather small amount of seasonal fluctuation. During the period of rapid vegetative growth, when presumably carbohydrates are being extensively oxidized, there is some increase in acidity. The absence of any strong buffer action is shown by the titration curves.

Data on the amount of dissolved matter in the sap of successive extractions of the leaves are given as a comment on the technic employed.

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A METHOD OF GROWING CORN SEEDLINGS (ZEA MAYS) FREE FROM SEED-BORNE ORGANISMS[1]

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In a previous paper[2] attention was called to the fact that corn seeds are almost universally infected with *Fusarium moniliforme* Sheldon. Subsequent studies have largely confirmed these results. Manns and Adams[3] have shown that several other organisms are frequently present in corn seeds which appear to be normal. Various germination tests have been recommended as simple means of selecting fungus-free seeds, and seeds so selected have been used, in some cases in critical experimental studies. In numerous tests conducted by the senior writer during the last five years, it has been determined that whereas the seeds of certain ears might appear to be free from fungi in germinator tests conducted for a period of six or eight days, and in culture dish tests, yet when grown in sterile sand for longer periods, the presence of *F. moniliforme* and some others of the common seed-borne organisms could usually be demonstrated. *Helminthosporium sp.* is rarely found in germination tests of corn seeds or in cultures made from crushed seed,[3] but if seedlings are grown in sand in an ordinary room and allowed to remain until they die, *Helminthosporium sp.* is quite commonly found fruiting on the stem and seed-coat remnants.

Because of the great difficulty in obtaining corn seed entirely free from fungi, for use in determining the pathogenicity of the numerous organisms associated with corn seeds and rotting roots, it has seemed necessary to develop a method by which corn seedlings might be freed completely from seed-borne organisms.

Sherbakoff[4] has developed a method by which he has been able to obtain seedlings 96 per cent free from organisms when grown in culture tubes. His determination was made, however, under very unnatural conditions, which might readily inhibit the development of fungi lodged between the thick seed-coat walls, whereas if the seedlings were grown in soil the organisms might develop, due to more favorable moisture conditions and breaking down of the seed coats by other microorganisms.

The present writers' attempts to duplicate the results obtained by Sherbakoff on corn seeds treated by his hot-water–bichloride method, have given results similar to his when the seeds were germinated on agar in test tubes. However, when they were germinated in sterilized sand in glazed jars the results were not so promising. Although the seeds and roots as a whole were much cleaner than those developed from seeds treated 20 minutes in 1 : 1,000 mercuric chloride solution,

[1] Received for publication Dec. 4, 1925; issued June, 1926.

[2] VALLEAU, W. D. SEED CORN INFECTION WITH FUSARIUM MONILIFORME AND ITS RELATION TO THE ROOT AND STALK ROTS. Ky. Agr. Expt. Sta. Bul. 226: 25–51, illus. 1920.

[3] MANNS, T. F., and ADAMS, J. F. PARASITIC FUNGI INTERNAL OF SEED CORN. Jour. Agr. Research 23: 495–524, illus. 1923.

[4] SHERBAKOFF, C. D. REPORT OF THE PLANT PATHOLOGIST. Tenn. Agr. Expt. Sta. Ann. Rpt. (1922) 35: 14–19. [1923.]

seed-coat discolorations and rotting roots were too frequent to warrant the use of this means of seed sterilization for critical studies of the pathogenicity of the organisms associated with seeds and rotting roots of corn plants.

A method has been developed by the writers which has proved practical and very efficient for greenhouse pot-culture work. Plants have been grown nearly to maturity, both in sand and soil cultures, which showed, on careful examination, the presence of no dead rootlets anywhere in the root system. This note is published in the hope

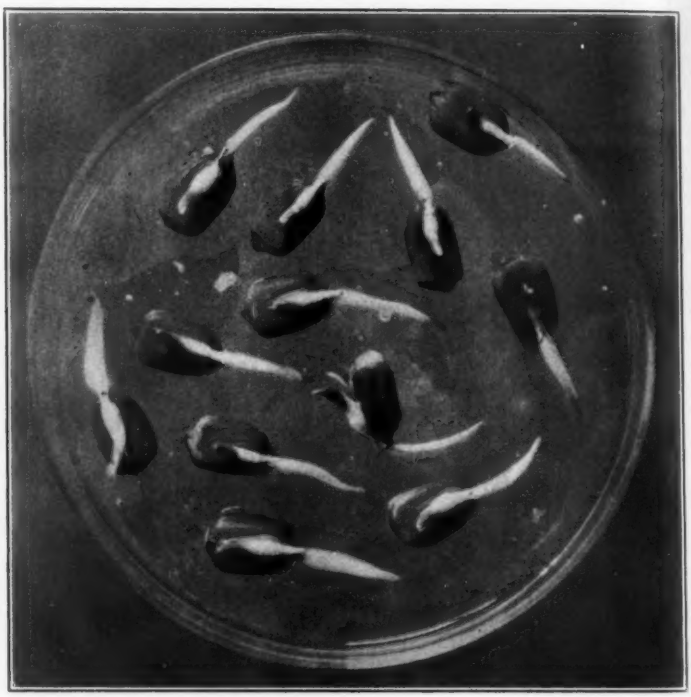

FIG. 1.—Following treatment in lime water, the seeds are placed in a culture dish for germination. The seed in the center turned over on its side is at the proper stage for removal of the seminal roots

that the method may be of value to others who are conducting critical studies of the effects of various organisms on the roots of corn plants.

The method is as follows: Seeds of any desired strain of corn are soaked in a mixture consisting of 1 part CaO to 4 parts of water. The seeds are shaken up in this and allowed to stand for about 20 hours. This is followed by 15 minutes in a 1 : 1,000 solution of mercuric chloride, after which the seeds are washed in sterile water.[5]

[5] Seed treatment with lime-water–bichloride has been nearly as efficient as Sherbakoff's method in apparently freeing seeds from organisms when treated seeds are tested only in test tubes of agar; but when the seedlings are grown in sterile sand it is found that seeds thus treated develop more and larger discolorations on the seeds coats and have a higher percentage of rotting rootlets in the neighborhood of the seed than when treated with the hot-water–bichloride method. It has the advantage, however, that germination is stimulated, if anything, whereas it is often seriously retarded by the hot-water–bichloride treatment.

They are then placed in sterile culture dishes on agar for germination (fig. 1). When the radicle is about one-half inch long and the paired seminal roots are just developing, these roots are cut off close to the seed, and the seed are transferred to a tube containing about 10 cubic centimeters of nutrient agar. Here growth continues, but

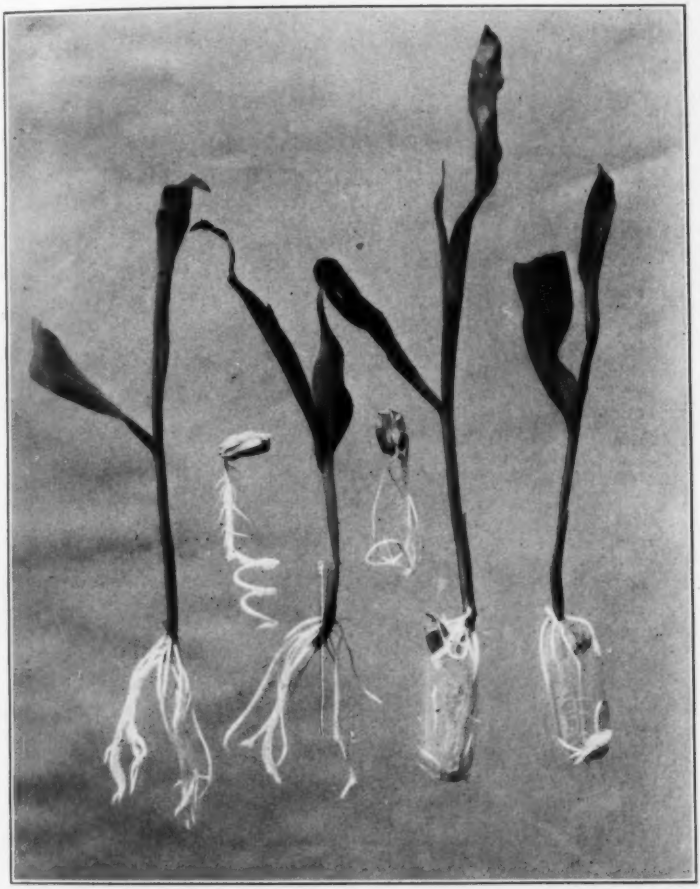

FIG. 2.—The seedlings at the right show the condition of the root system in the agar before removal of the seed. At the left are the seeds, and what remains of the seminal roots, after removal from the seedlings; and two of the seedlings from which the seeds were removed, ready to be transplanted to soil or sand

instead of the seminal roots developing, roots are sent out from the internode of the epicotyl and from the first node. When these roots have penetrated an inch or more into the agar, the seedling is removed from the tube and, if it shows no signs of fungus infection, the seed is severed from the plant, thus leaving the seedling with a fairly

well-developed root system, but minus the seed and any fungi which it may harbor (fig. 2). It may then be transferred to the sand or soil cultures where it is to be grown. Here it should be shaded for

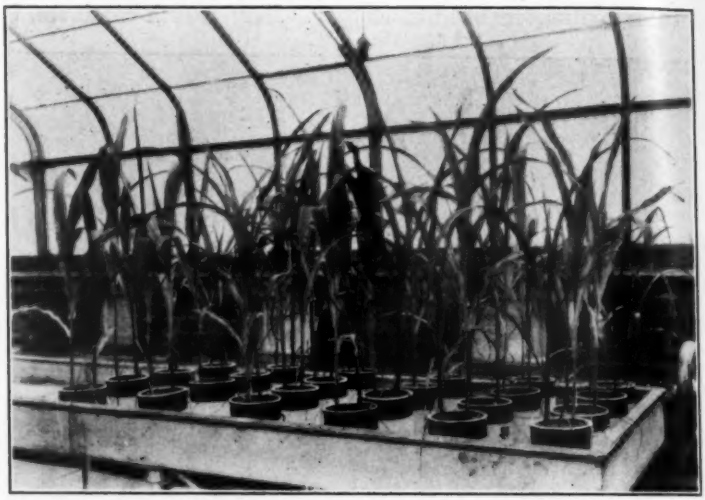

FIG. 3.—Forty-five-day-old plants in soil cultures, raised according to the method described in the text

two or three days until it becomes well established. If the roots are allowed to develop sufficiently in the test tube before the removal of the seed, the mortality due to transplanting is very slight.

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